



BIOCHEMISTRY OF FOOD SPOILAGE




Carbohydrate

Proteins

Energy

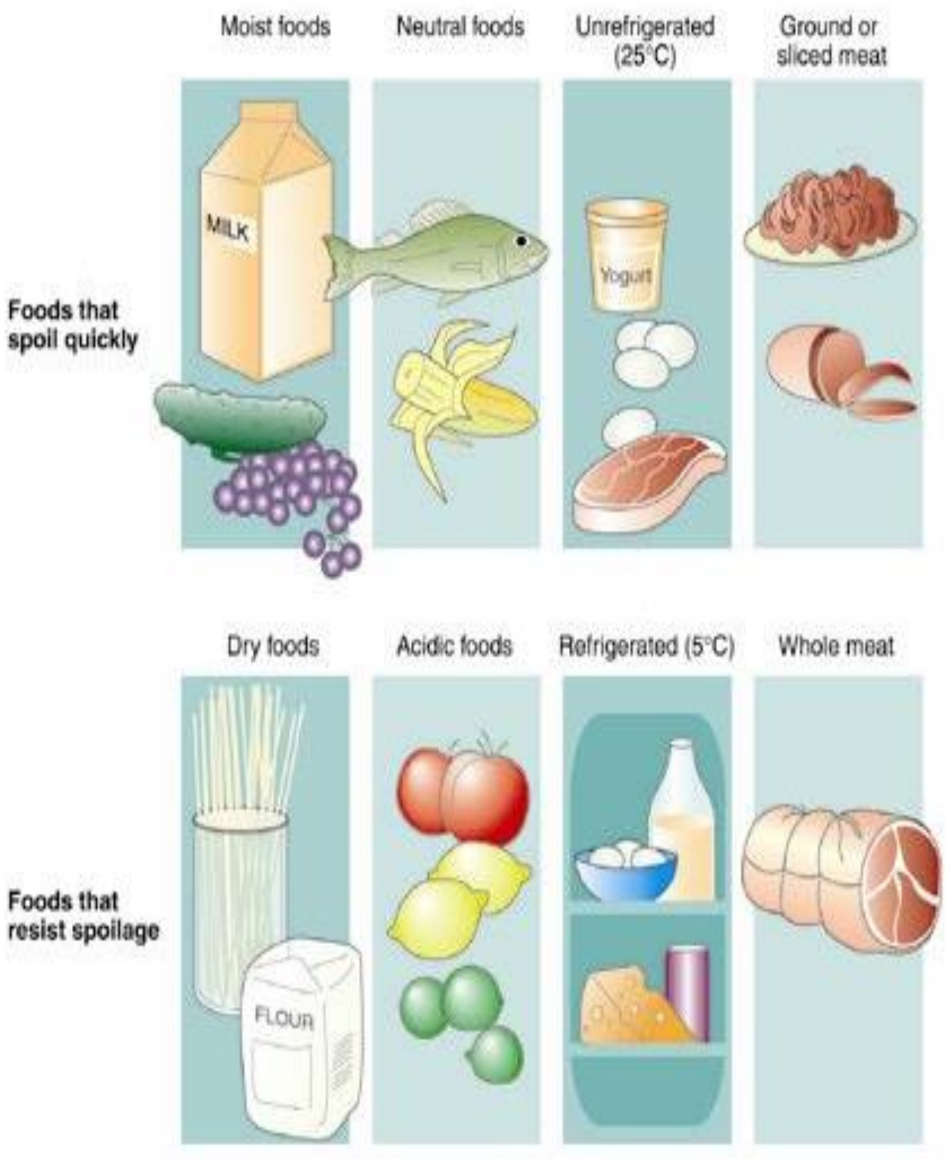
Building materials

Vitamins

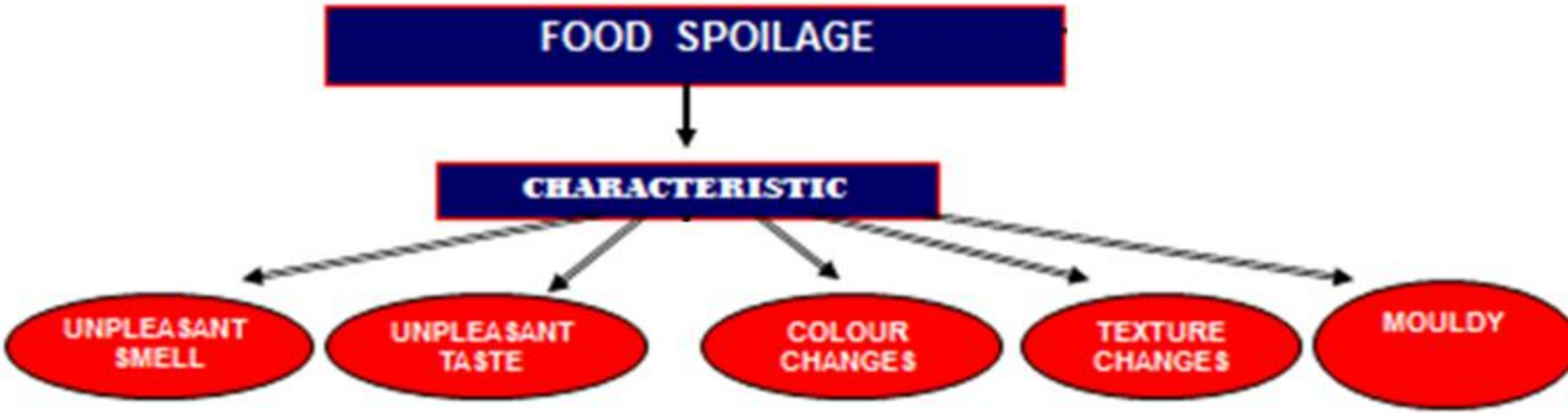
Lipids

Human Growth

FOOD GROUPS



- Highly Perishable
  - Meat
  - Fruit
  - Milk
  - Vegetables
  - Eggs
- Semi perishable
  - Potatoes
  - Nuts
  - Flour
- Stable
  - Rice
  - Dry beans


FOOD SPOILAGE
CHARACTERISTIC
UNPLEASANT SMELL
UNPLEASANT TASTE
COLOUR CHANGES
TEXTURE CHANGES
MOULDY


Food
Food deterioration
Food spoilage
Economic loss

Biochemistry of food spoilage

Substrates

Chemical reactions

Chemical compounds

Factors

Major causes of food spoilage

Physical

- Temperature
- R.H.
- Light
- Mechanical damage

Chemical

- Enzymatic reaction
- Non enzymatic reactions
- Rancidity
- Chemical interaction

Microorganisms

- Bacteria
- Yeast
- Molds

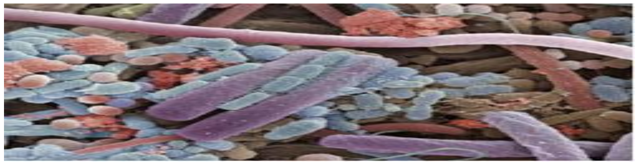
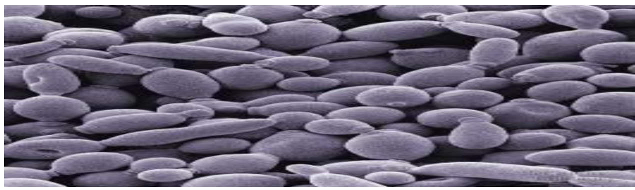
Others

- Insects
- Rodents
- Animals
- Birds



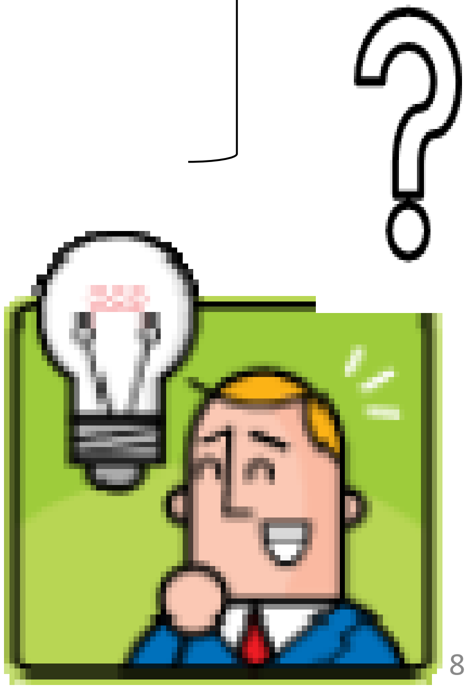




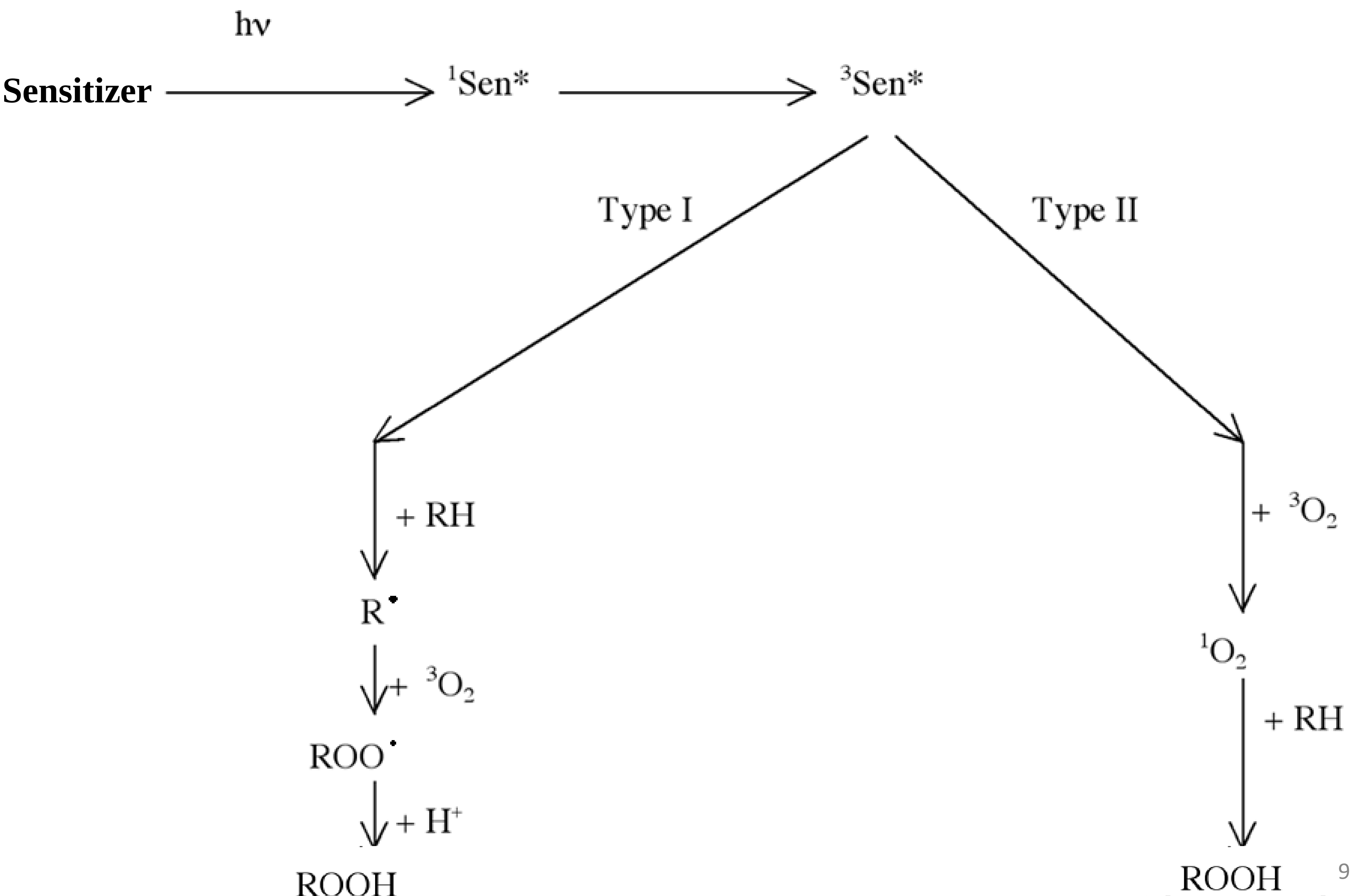



LIGHT

- **Oxidation of food**
  - ✓ Reversion flavor of soyabean
  - ✓ Sunlight flavor in milk
  - ✓ Rapid loss of Riboflavin, vitamin D, E and C
  - ✓ Greening of potato

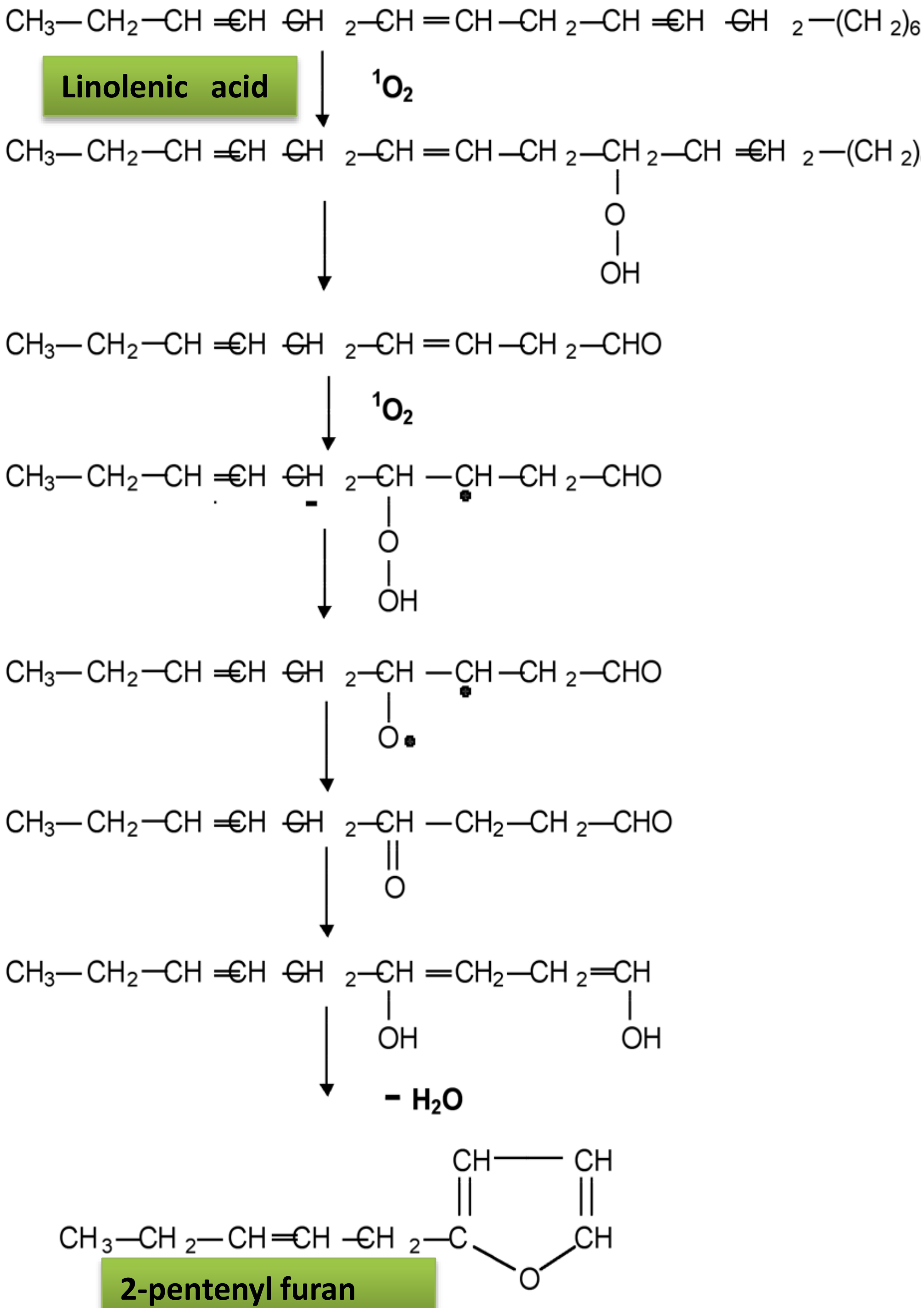


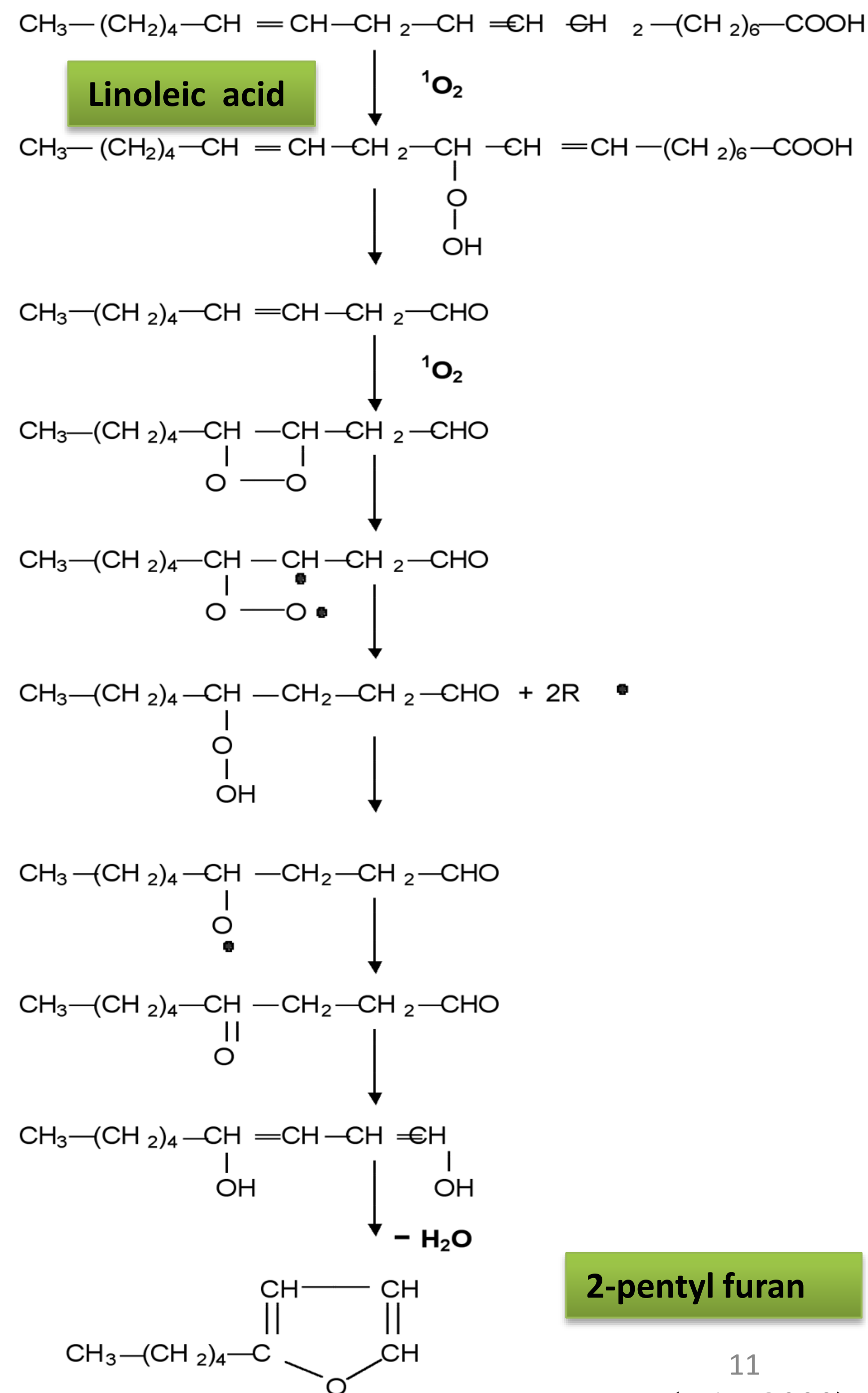
Formation of excited triplet sensitizer (3Sen*) and its reaction with substrate via Type I and Type II reactions



(Sharman *et al.*, 2000)

Reversion flavor in soybean oil


Linolenic acid
2-pentenyl furan


Linoleic acid
2-pentyl furan

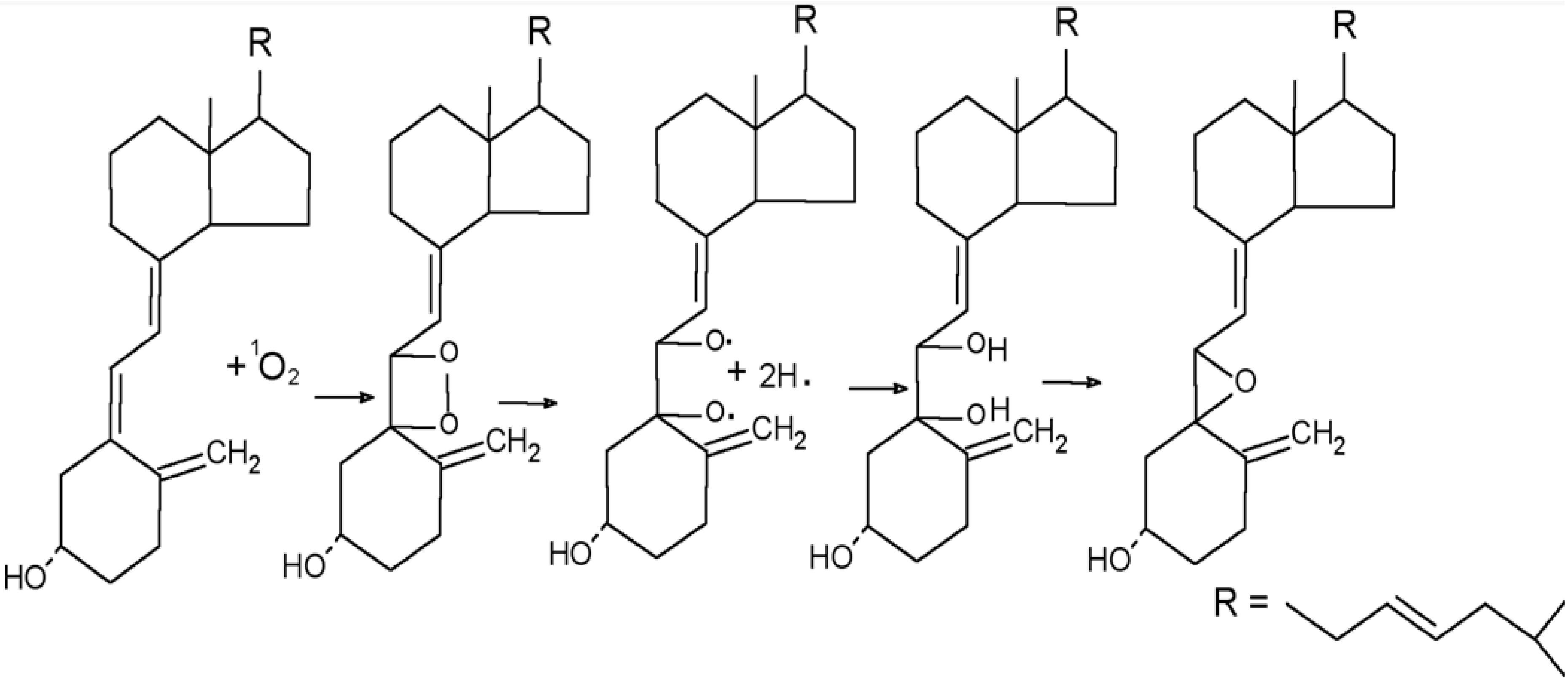
(Min, 2000)

Effect of chlorophyll on pentenyl furan peak areas

Light Exposure(d)	Added Chlorophyll		
	0 ppm	1 ppm	5 ppm
1	0	0	0
2	0	0	0
3	0	0	1502
4	0	1534	3018

(Callison, 2001)

Riboflavin Photosensitized Singlet Oxygen Oxidation of Vitamin D



Vitamin D

vitamin D-5,6 epoxide

(King, 1996)

Head space oxygen of vitamin D_2 samples with 15 ppm riboflavin stored in the dark from 1-8 hours

Time (h)	Vitamin D2 (ppm)	Headspace oxygen remaining* (%)
1	3000	21.40
2	3000	21.54
4	3000	21.60
8	3000	21.55
1	9000	21.32
2	9000	21.19
4	9000	21.19
8	9000	20.61

* no significant change with time

(King and Min, 1998)

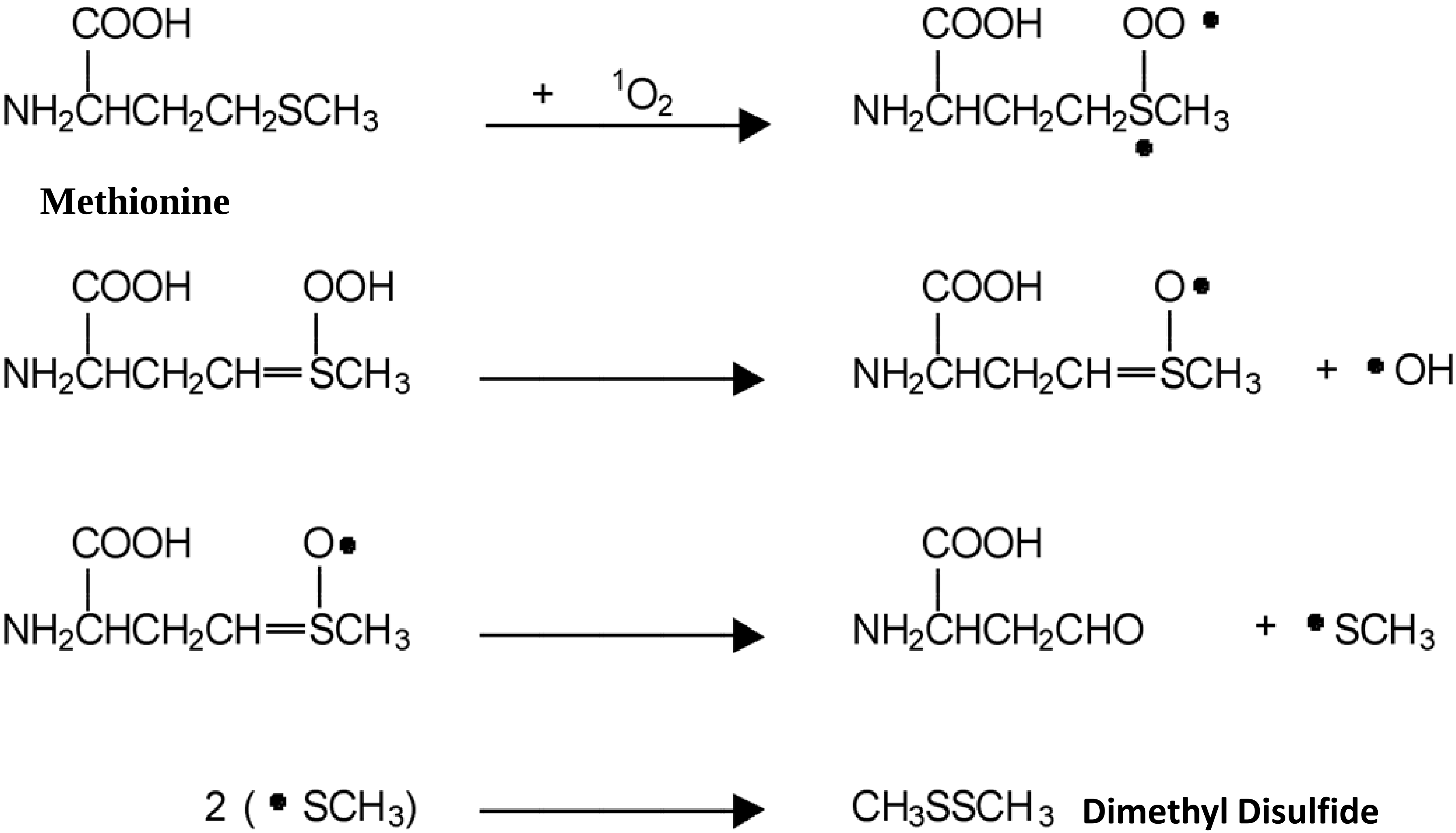
Effect of vitamin D2 and riboflavin concentrations on % headspace oxygen loss during storage in the light from 1 to 8 hours

Time (h)	Vitamin D2 (ppm)	Riboflavin (ppm)	Headspace oxygen loss* (%)
1	3000	5	3.71
2	3000	5	5.55
4	3000	5	7.15
8	3000	5	9.56
1	6000	5	6.91
2	6000	5	10.0
4	6000	5	15.5
8	6000	5	26.2
1	9000	5	8.95
2	9000	5	13.8
4	9000	5	22.0
8	9000	5	39.6
1	3000	10	6.14
2	3000	10	9.15
4	3000	10	13.0
8	3000	10	17.1
1	6000	10	9.77
2	6000	10	15.6
4	6000	10	22.4
8	6000	10	35.1
1	9000	10	12.6
2	9000	10	23.2
4	9000	10	32.6
8	9000	10	53.2
1	3000	15	9.37
2	3000	15	14.2
4	3000	15	18.6
8	3000	15	26.5
1	6000	15	13.1
2	6000	15	19.8
4	6000	15	29.5
8	6000	15	45.2
1	9000	15	16.4
2	9000	15	25.1
4	9000	15	38.8
8	9000	15	57.3

* significant change with time at $p<0.05$

(King and Min, 1998)

Singlet Oxygen and Ascorbic Acid Effects on Dimethyl Disulfide and Off-Flavor in Skim Milk Exposed to Light



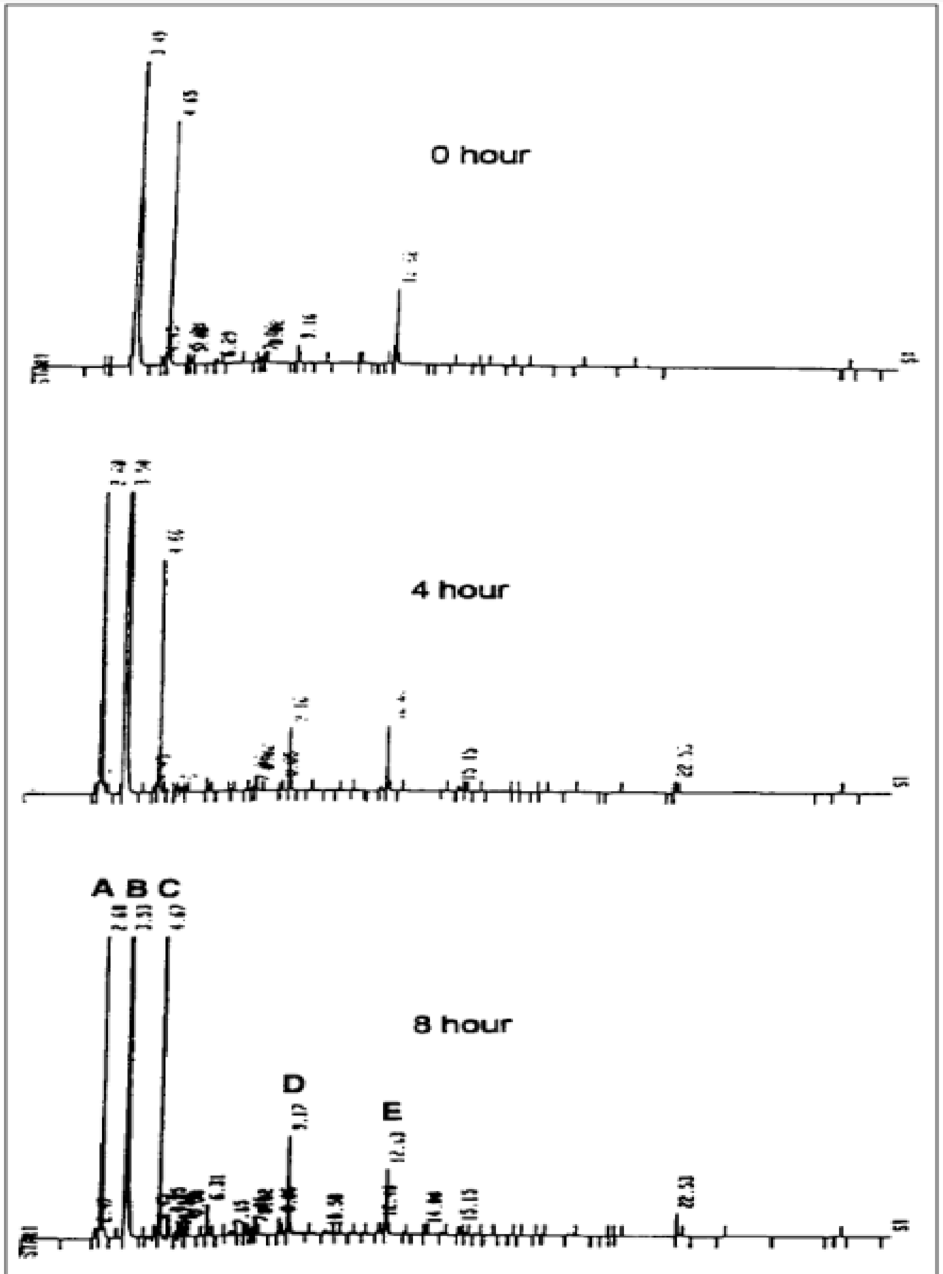
Methionine

Dimethyl Disulfide

Postulated mechanism of dimethyl disulfide formation by singlet oxygen oxidation of methionine.

(Jung *et al*., 1998)

Effects of time of exposure to fluorescent light on headspace volatile compounds and dimethyl disulfide of skim milk



A - 2-butanone

B - ethanol

C - diacetyl

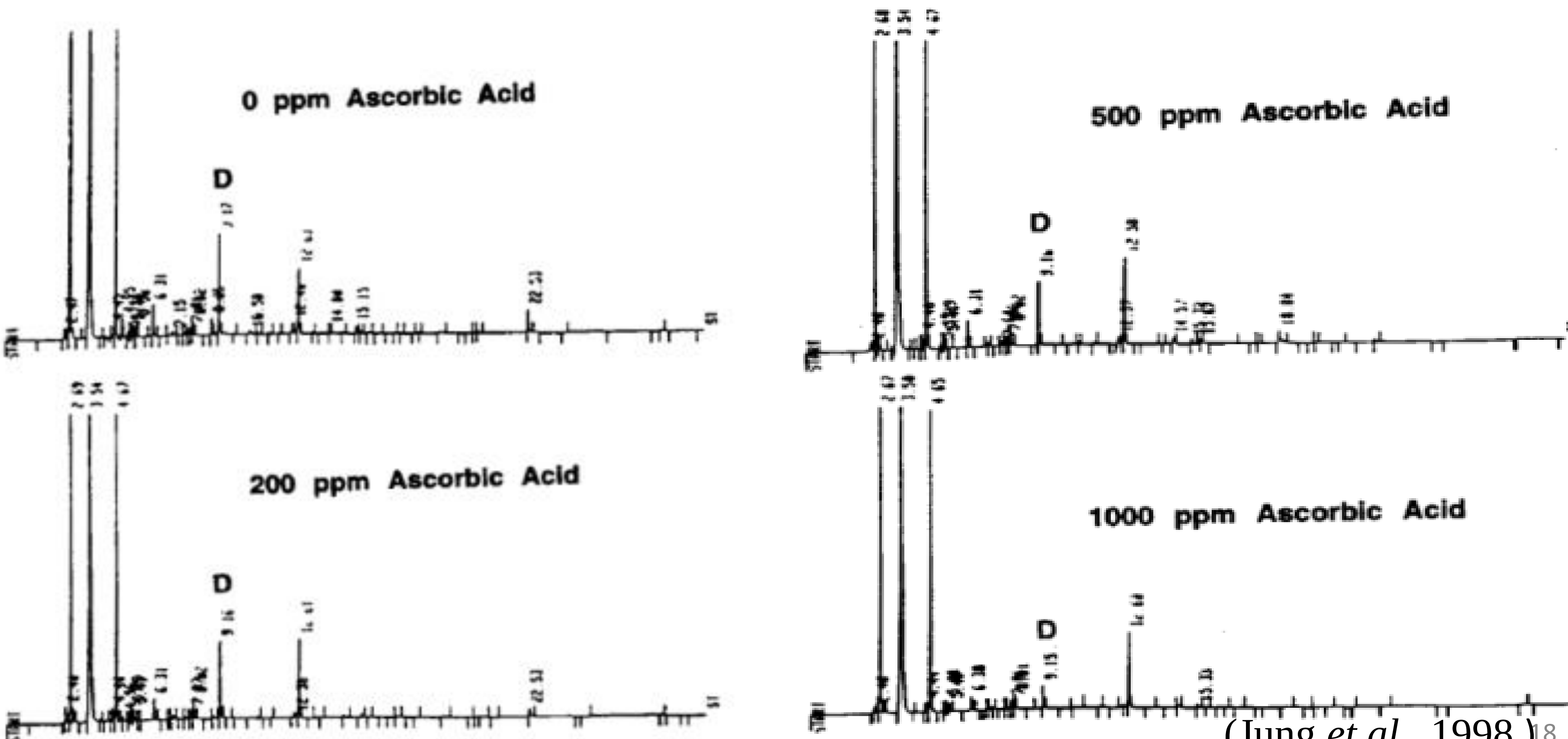
D - dimethyl disulfide

E - n-butanol

(Jung *et al*., 1998)

Effects of ascorbic acid concentration on dimethyl disulfide (peak D) content in skim milk during light exposure for 1h.

Ascorbic acid (ppm)	Dimethyl Disulphide (peak D)
0	13269
200	6158
500	4907
1000	4742



(Jung *et al.*, 1998)

Greening of potato

+ **Light** →

Biosynthesis of chlorophyll

↓

Fixation of carbon dioxide

↓

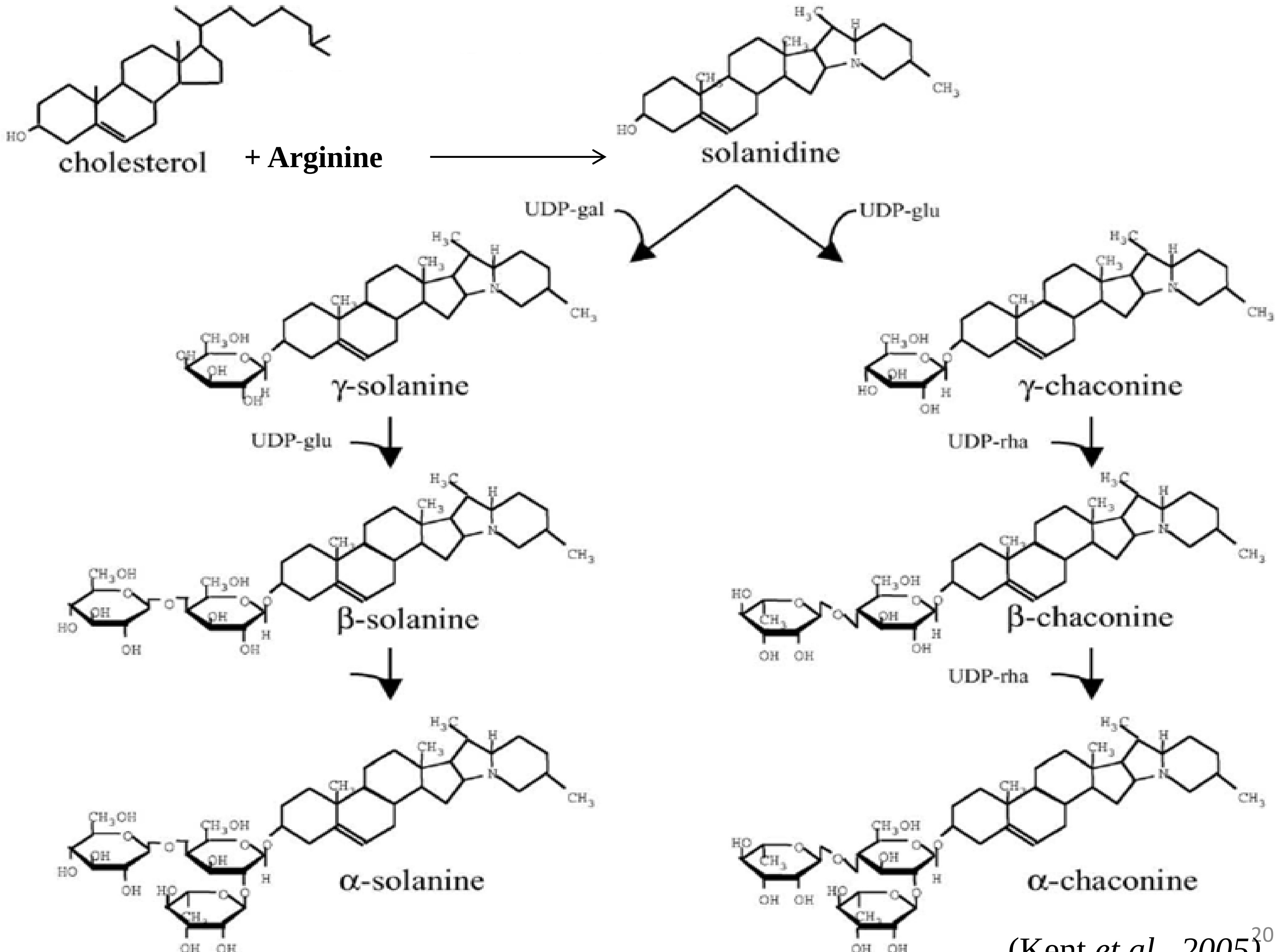
Acetate

↓

Mevolenic acid

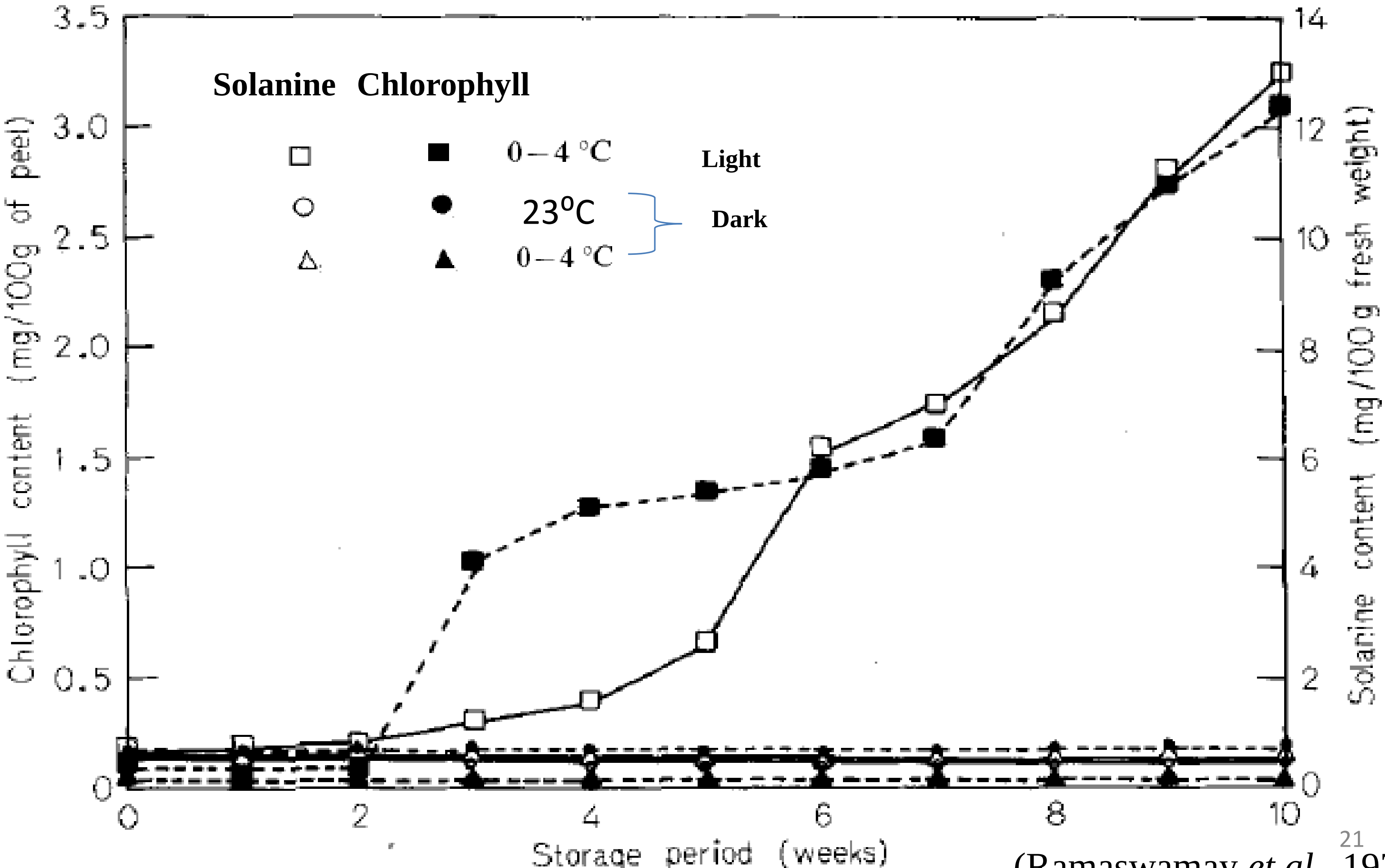
↓

Cholesterol



(Kent *et al., 2005)*

Chlorophyll and solanine synthesis in potatoes

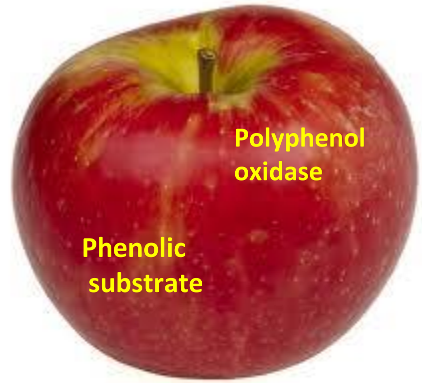


(Ramaswamay *et al.*, 1976)

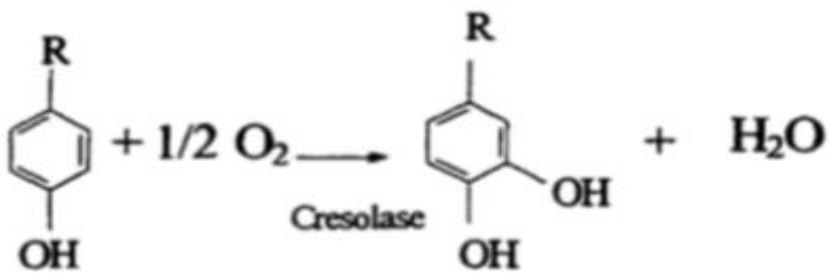
Enzymes that cause food spoilage

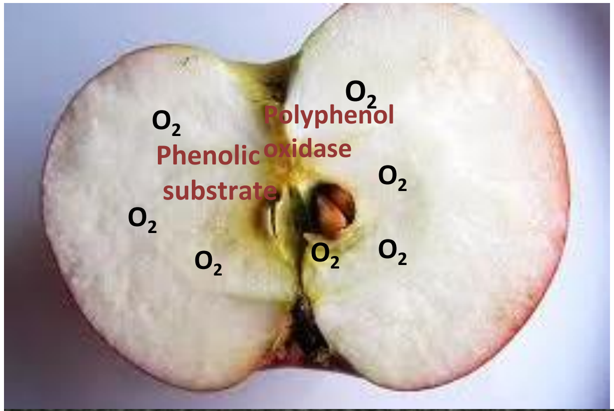
Enzymes	Food	Spoilage action
Ascorbic acid oxidase	Vegetables	Destruction of vitamin C
Lipase	Milk, oils	Hydrolytic rancidity
Lipoxygenase	Vegetables	Destruction of vitamin A
Pectic enzymes	Fruits	Destruction of pectic substances (Softening)
Peroxidases	Fruits	Browning
Polyphenoloxidase	Fruits, vegetables	Browning, off flavour, vitamin loss
Proteases	Eggs crab, lobster Flour	Reduction of shelf life Overtenderization Reduction in gluten network formation
Thiaminase	Meats, fish	Destruction of thiamine

ENZYMATIC BROWNING

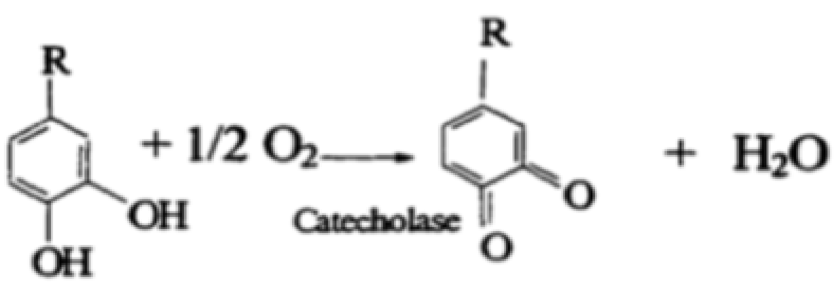


- Hydroxylation of monophenol to *o*-diphenol

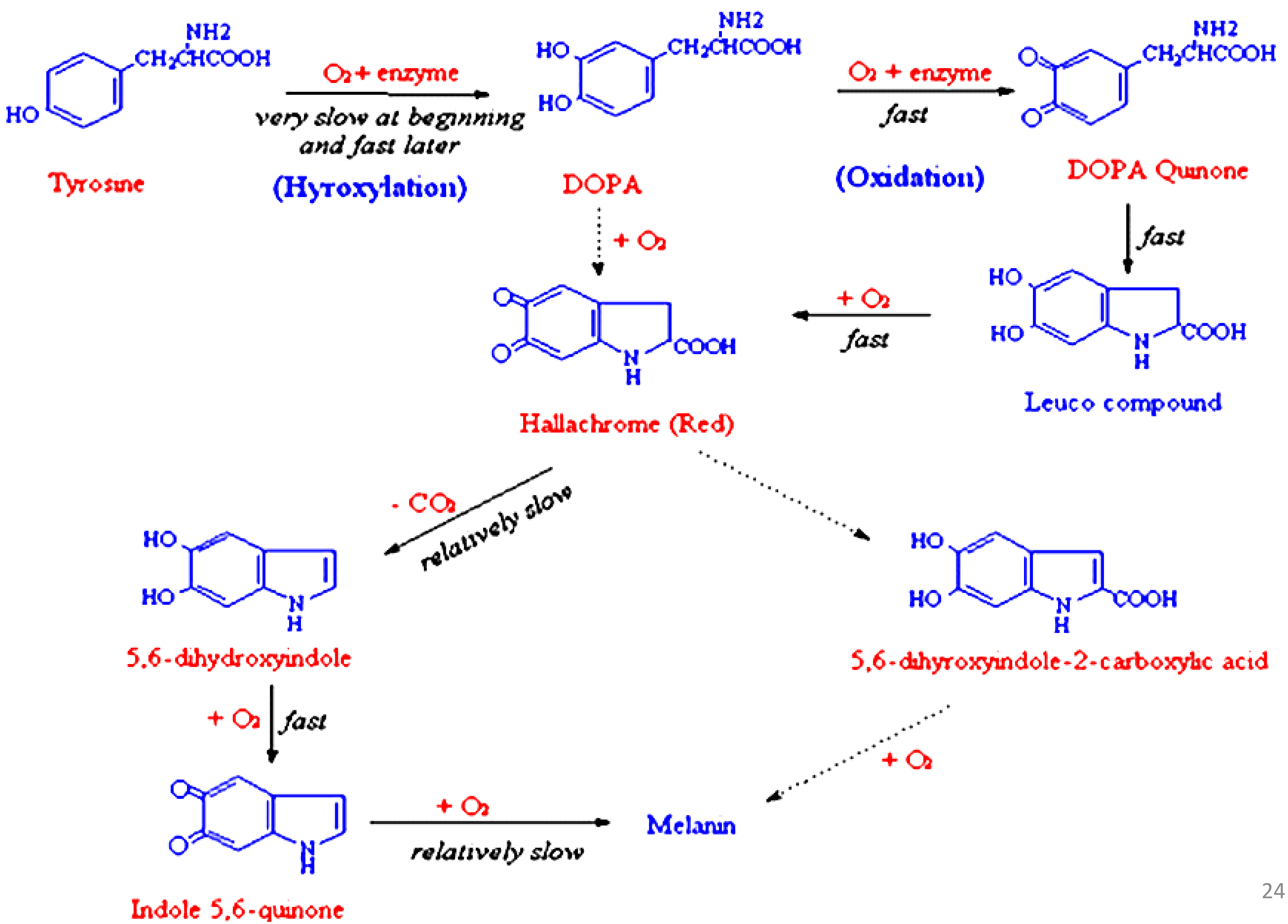




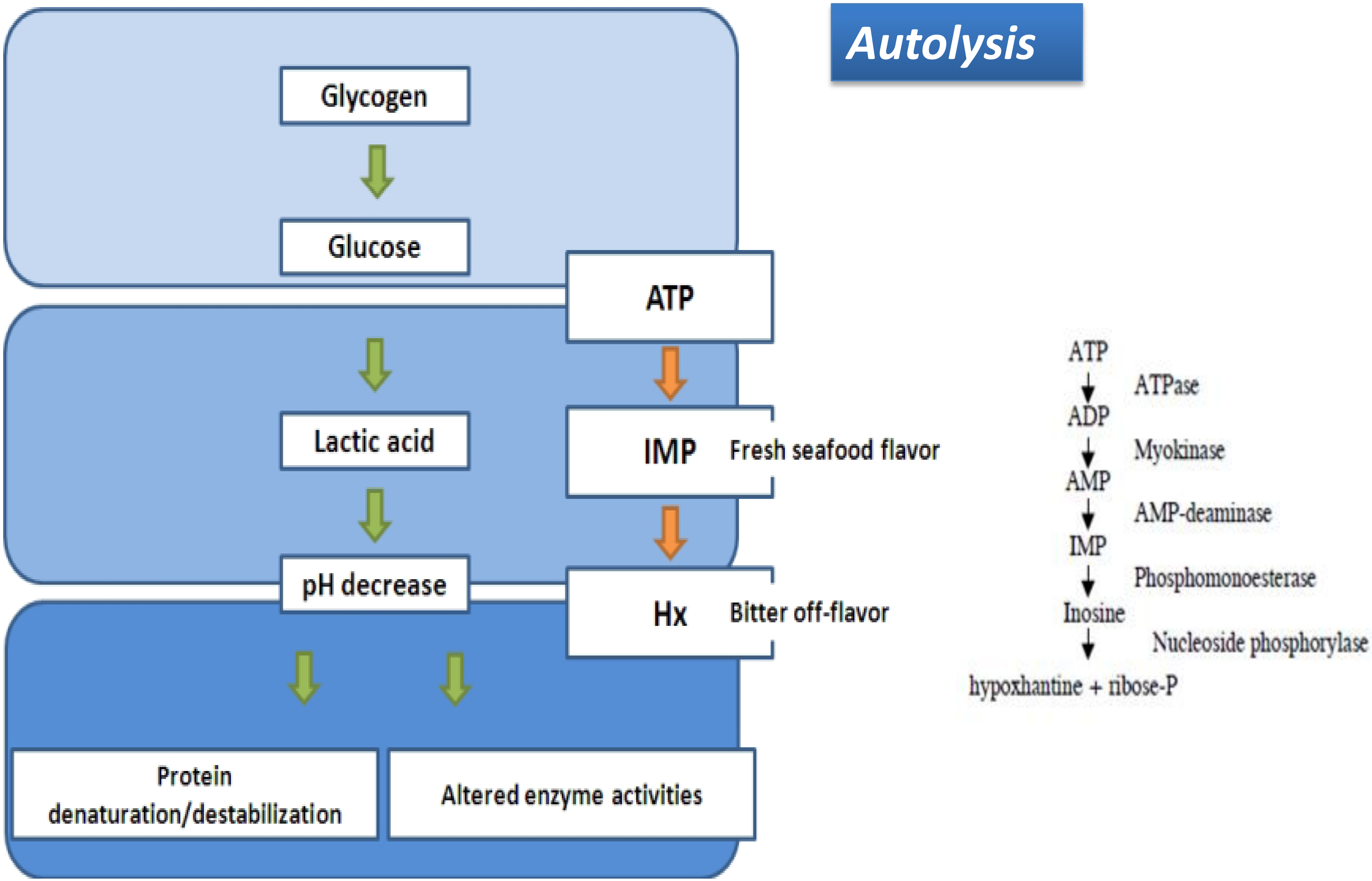
- Dehydrogenation of *o*- diphenol to *o*-quinone



Oxidation of tyrosine by Phenolase and the formation of melanin pigment



Post-mortem changes in fish muscle due to autolytic degradation

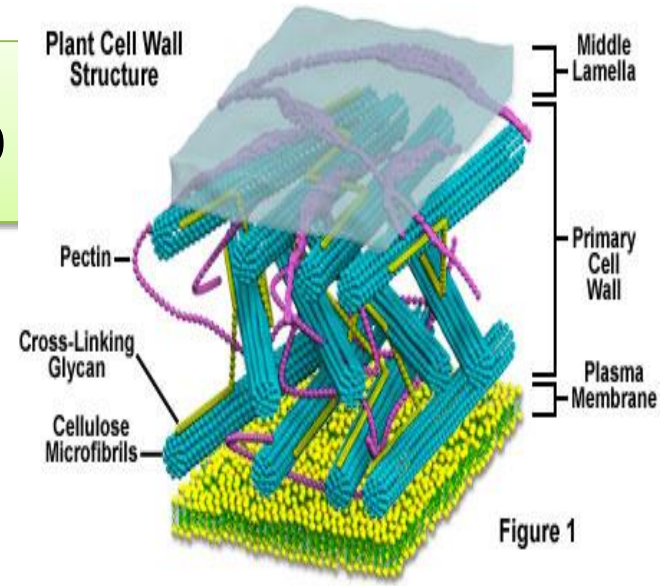


(Green, 2011)

An overview of fruit ripening with particular emphasis on textural softening

Protopectin Decomposition → Soluble Pectin → Softening (over matured fruit)





Unripe → Intermediate → Ripe

Unripe ↓

SAM
↓ ACC synthase
ACC
↓ ACC oxidase
Ethylene

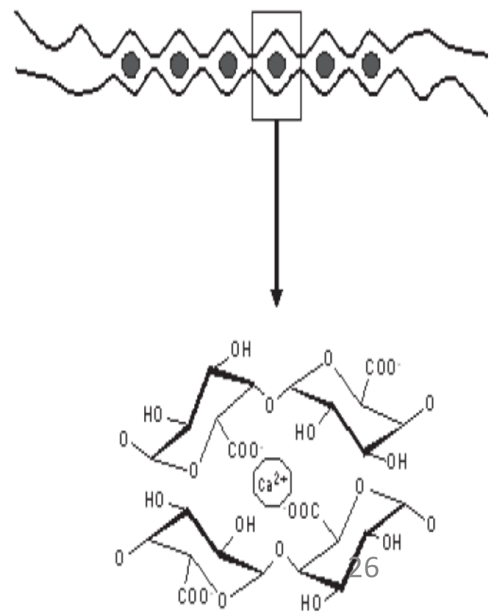
→ Physiological Biochemical Molecular Organoleptic Changes → Intermediate

PG, PME & Other CHO-Hydrolases → Edible fruit with desirable color, flavor and texture

Ripe ↓ Edible fruit with desirable color, flavor and texture

↓ Senescence Excessive textural softening

↓ Spoilage/loss

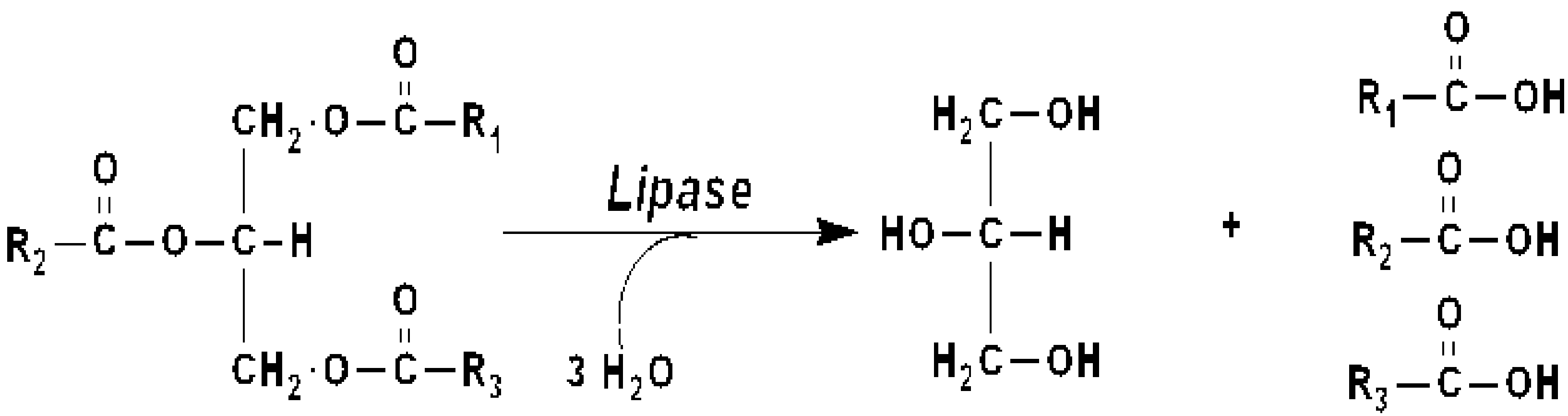


RANCIDITY

Hydrolytic Rancidity

Oxidative Rancidity

Hydrolytic Rancidity



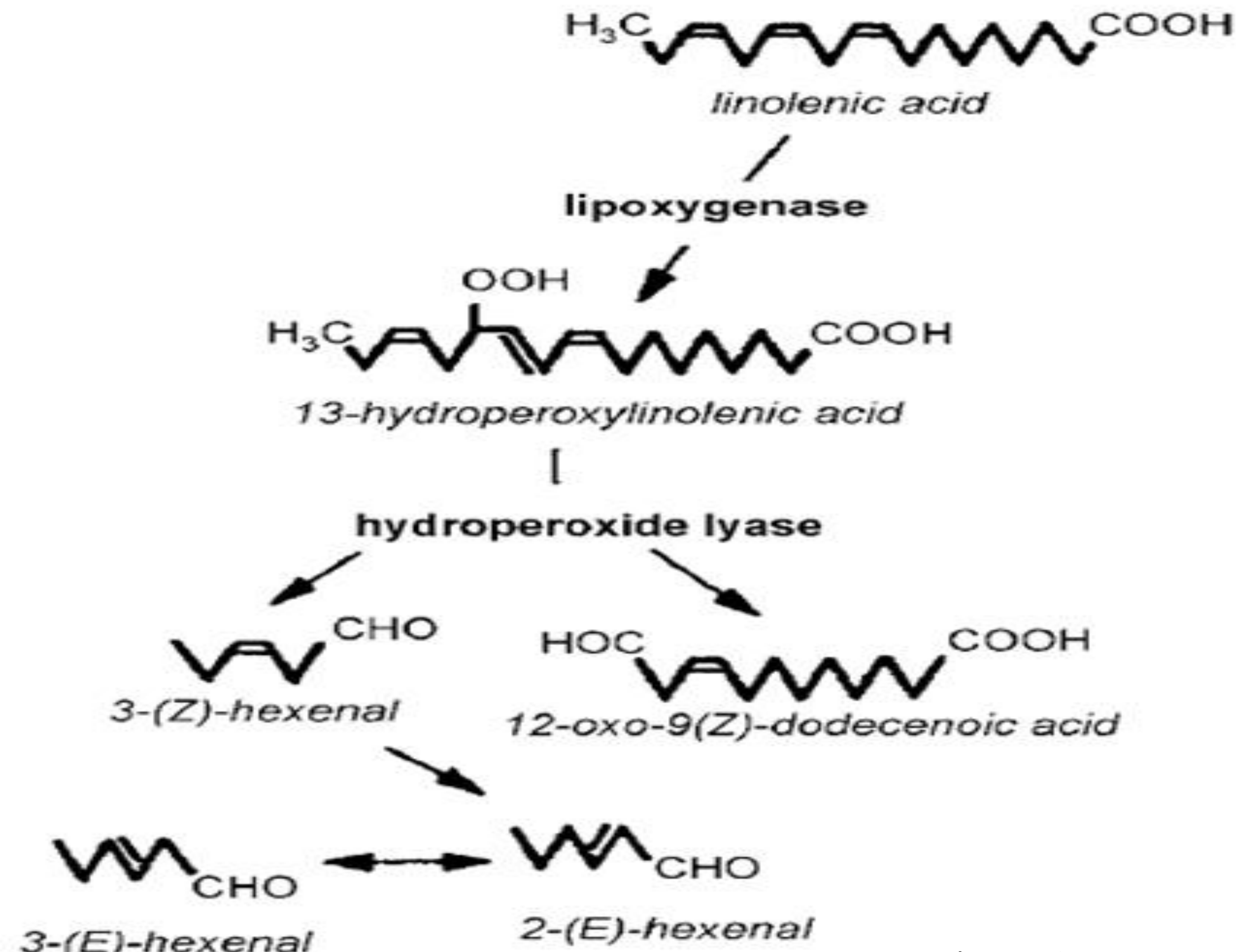
Triacylglycerol

Glycerol

Free fatty acids

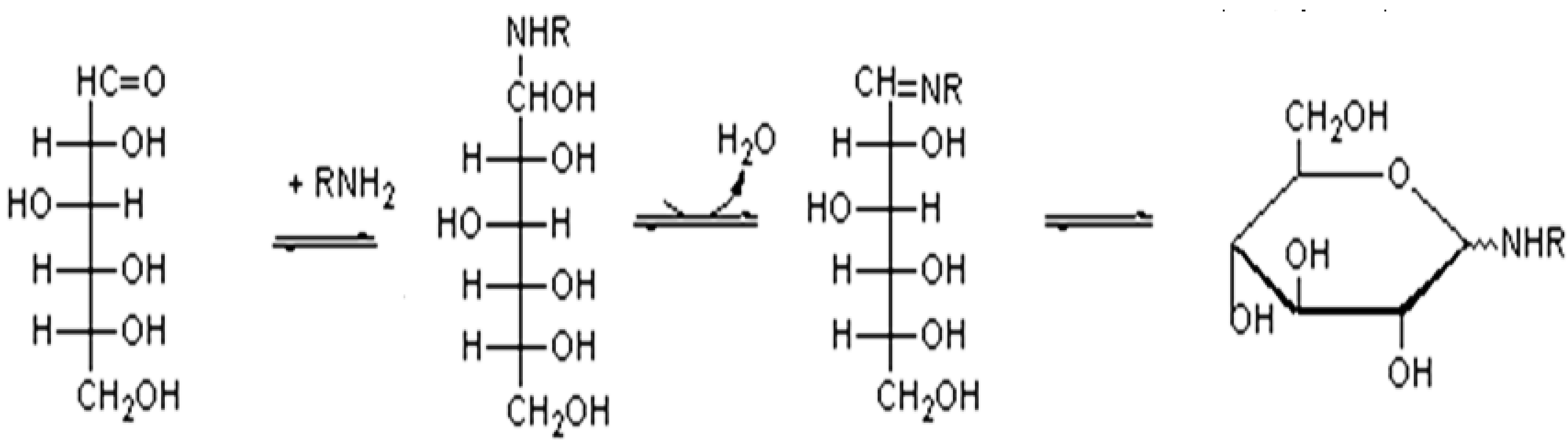
(Volatile bad odor)

Oxidative Rancidity

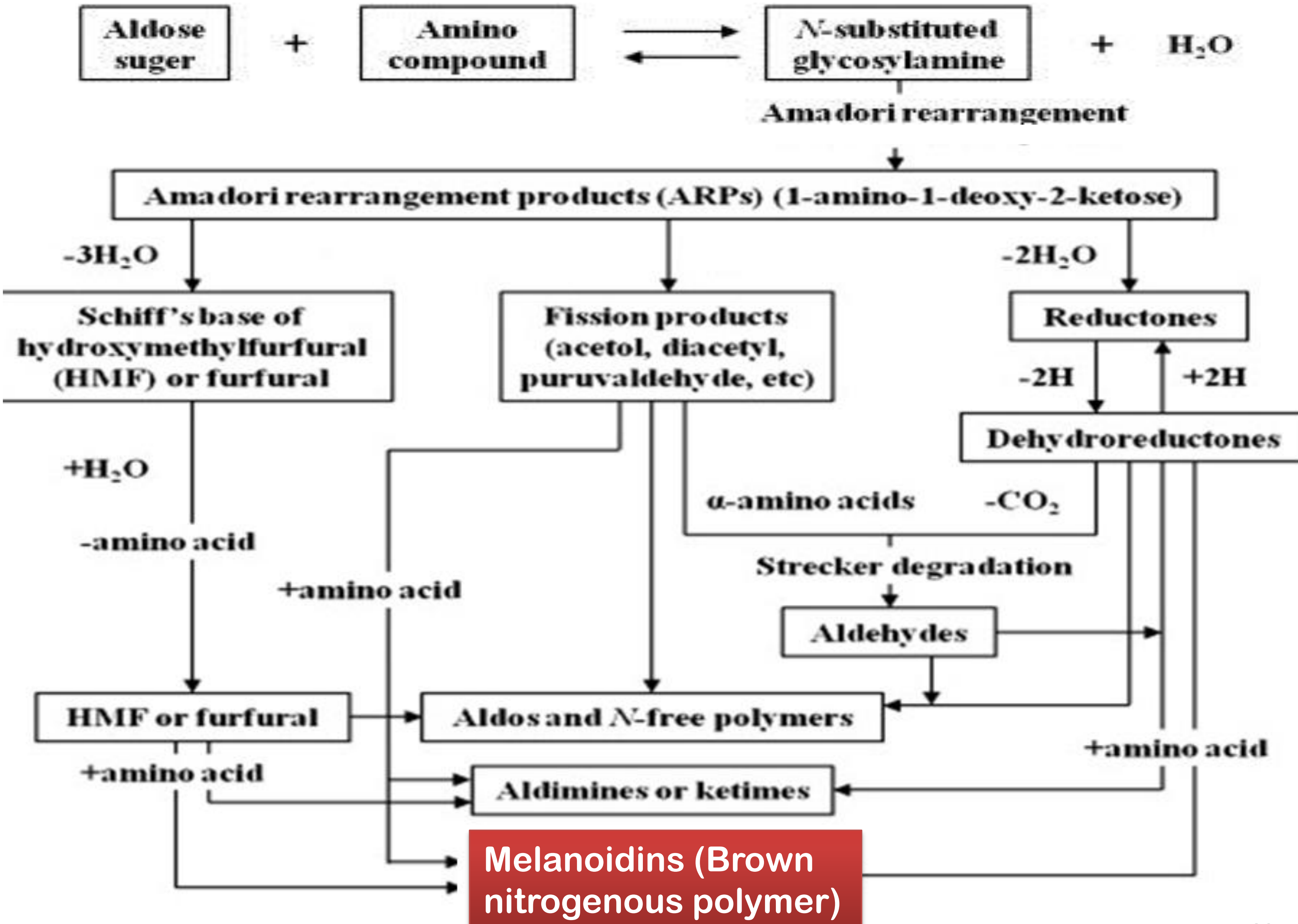


(Baysal and Demirdoven, 2006)

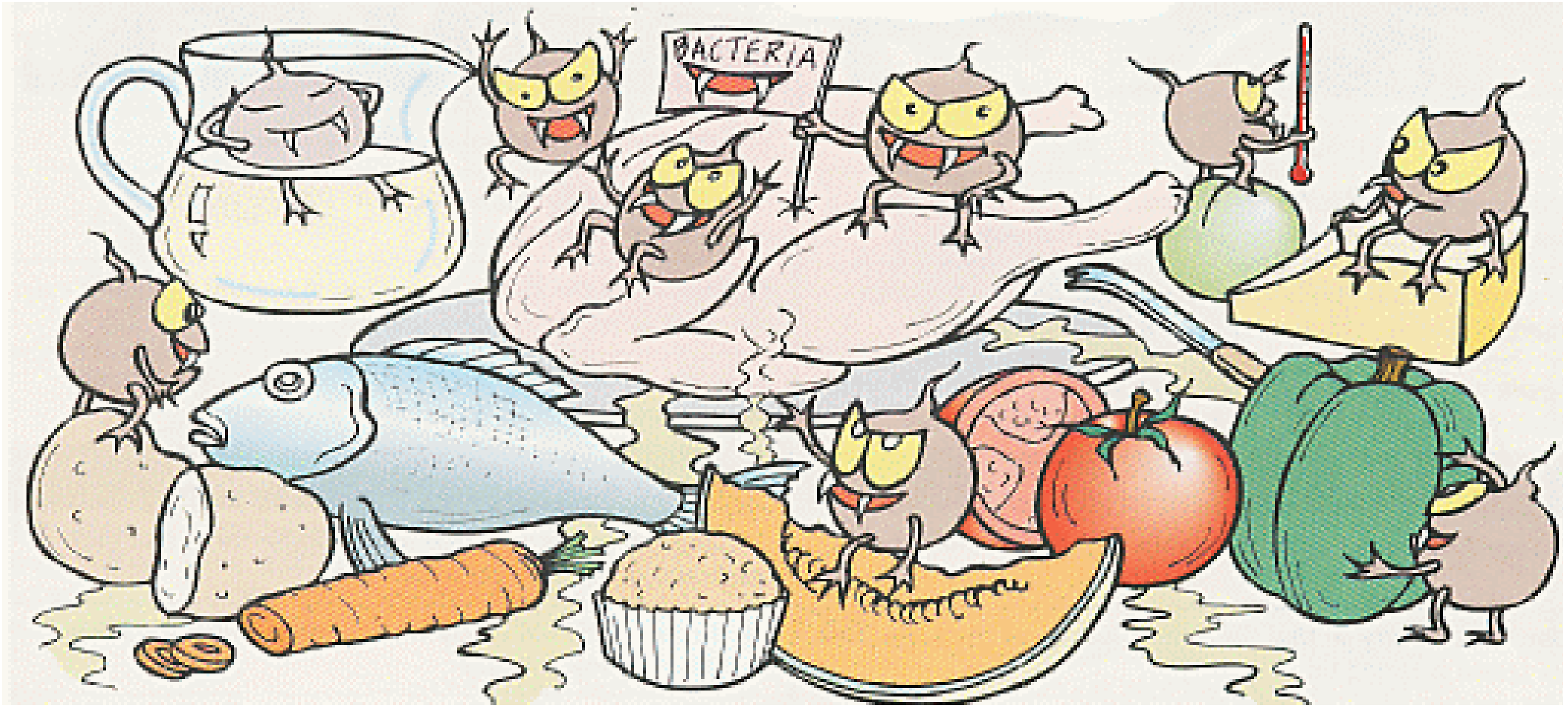
MAILLARD REACTION

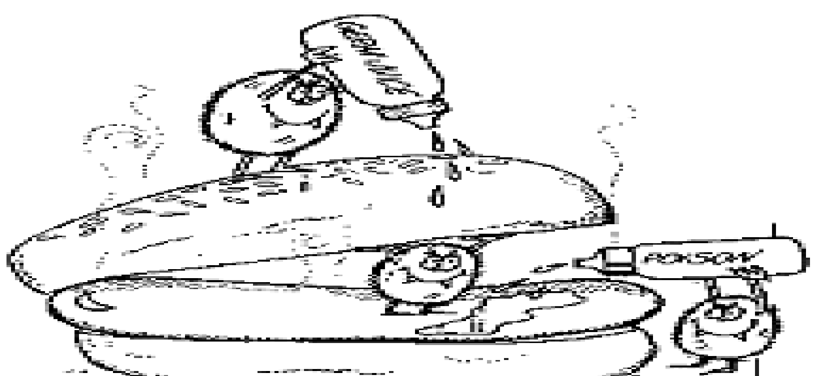


D-glucose **Schiff Base** **Glucosamine**


Aldose suger
+
Amino compound
N-substituted glycosylamine
+
H_2O
Amadori rearrangement
Amadori rearrangement products (ARPs) (1-amino-1-deoxy-2-ketose)
$-3H_2O$
$-2H_2O$
Schiff's base of hydroxymethylfurfural (HMF) or furfural
Fission products (acetol, diacetyl, puruvaldehyde, etc)
Reductones
-2H
+2H
Dehydroreductones
$+H_2O$
-amino acid
α-amino acids
$-CO_2$
Strecker degradation
+amino acid
Aldehydes
HMF or furfural
Aldos and N-free polymers
+amino acid
+amino acid
Aldimines or ketimes
Melanoidins (Brown nitrogenous polymer)

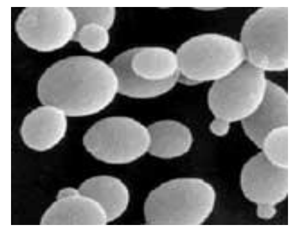
Microbial Spoilage



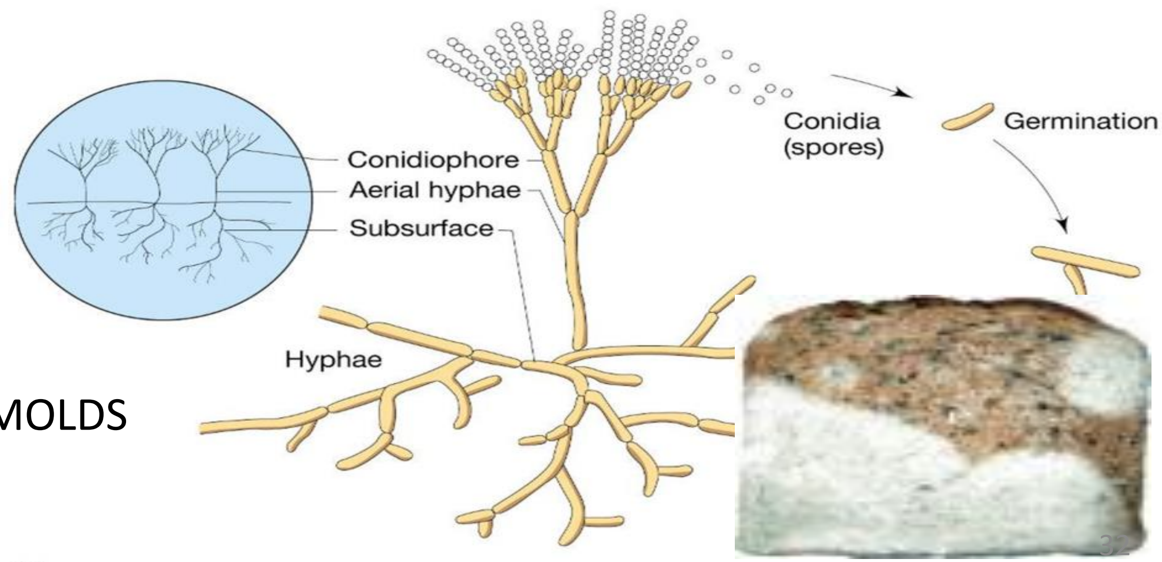


BACTERIA

Microbial Spoilage



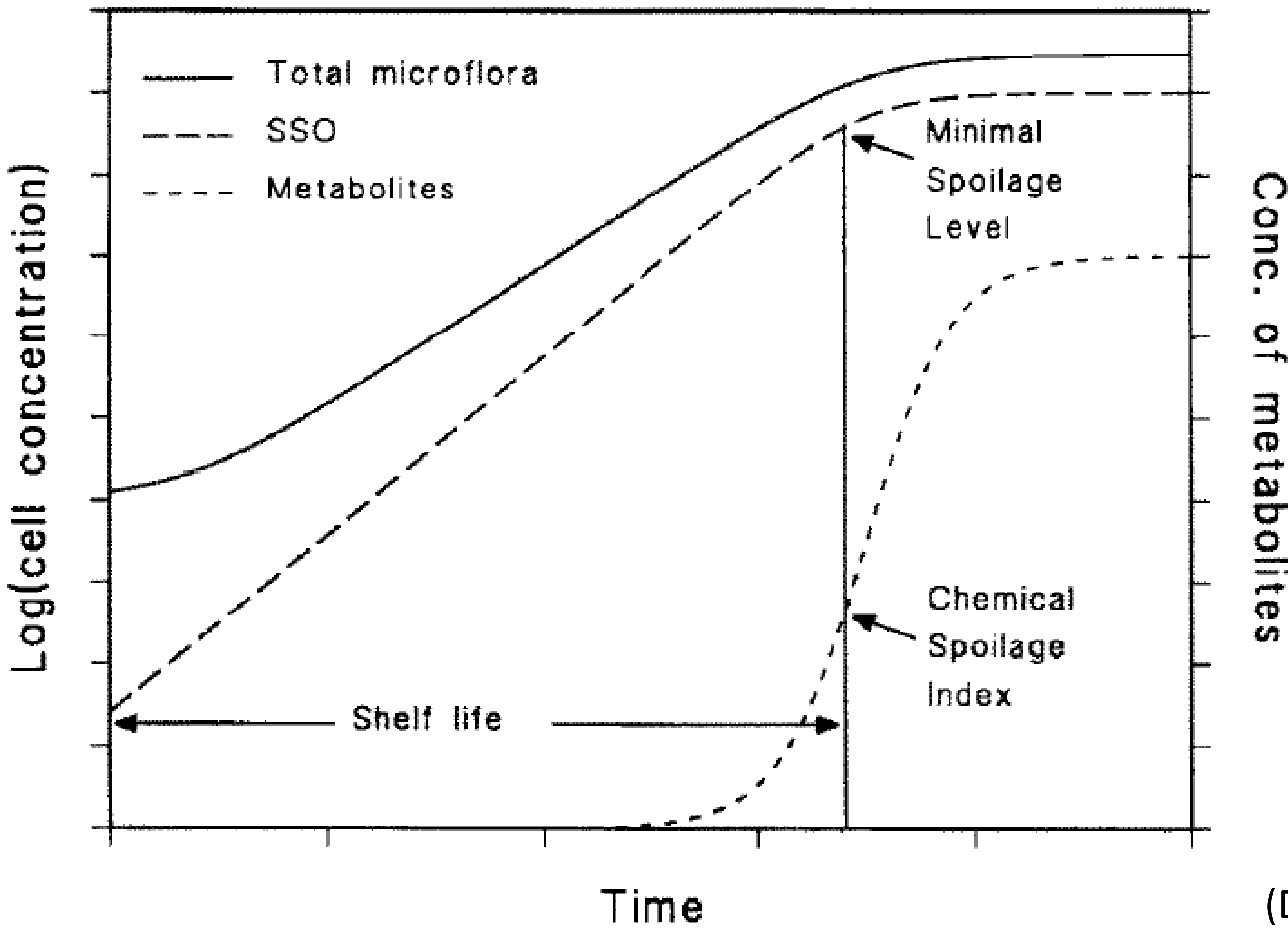
YEAST



MOLDS

(b)

General pattern of microbial spoilage



(Dalgaard, 1993)

Factors affecting growth of microbes

- Intrinsic
  - ✓ Nutrients
  - ✓ a_w
  - ✓ pH
  - ✓ Redox potential
  - ✓ Inhibitors

- Extrinsic
  - ✓ Temperature
  - ✓ Humidity
  - ✓ Atmosphere

- Implicit
  - ✓ Interactions of microorganisms

Fungal spoilage of starch-based foods in relation to its water activity

Days before visible appearance of fungi on six starch-based food at 25°C. Average of three determinations

a_w	Rice	Glutinous rice	Rice flour	Glutinous rice flour	Wheat flour	Corn flour
0.98	7 ± 2	7 ± 2	11 ± 3	14 ± 2	8 ± 1	22 ± 3
0.95	9 ± 1	9 ± 2	15 ± 4	17 ± 4	10 ± 1	51 ± 2
0.90	10 ± 2	10 ± 3	20 ± 1	24 ± 3	10 ± 1	69 ± 2
0.85	10 ± 2	10 ± 0	27 ± 1	39 ± 2	14 ± 3	124 ± 4
0.80	13 ± 1	17 ± 1	28 ± 3	64 ± 4	17 ± 1	[a]
0.75	20 ± 2	19 ± 1	32 ± 1	91 ± 3	27 ± 2	[a]
0.65	57 ± 2	73 ± 1	[a]	[a]	[a]	[a]

[a] No fungal development at 6 months incubation.

(Abdullah *et al*., 2000)

MICROBIAL SPOILAGE – HOW DOES IT MANIFEST ITSELF?

- Visible growth
- Gas production
- Slime
- Enzymes
- Off-flavours

Food		Types of Spoilage	Spoilage Microorganisms
MEAT	Fresh	Putrefaction	*Clostridium*, *Pseudomonas*, *Proteus*, *Alcaligenes*, *Chromobacterium*
		Souring	*Chromobacterium*, *Lactobacillus*, *Pseudomonas*
	Cured	Mouldy	*Penicillium*, *Aspergillus*, *Rhizopus*
		Souring	*Pseudomonas*, *Micrococcus*, *Bacillus*
		Slimy	*Leuconostoc*
	Vacuum Packed	Souring Greening	*Lactobacillus*, *Carnobacterium*, *Leuconostoc*
Poultry		Odor, Slime	*Pseudomonas*, *Alcaligenes*, *Xanthomonas*

Food	Types of Spoilage	Spoilage Microorganisms
DAIRY	Bitterness	*Pseudomonas spp.*
	Souring	*Lactobacillus thermophilus*
	Sweet curdling	*Bacillus cereus*
	Green discoloration	*Penicillium*
	Green to black discoloration	*Cladosporium*
	Black discoloration	*Candida*
	Sliminess (high pH)	*Pseudomonas spp.*
	"Gassy" cheese	*Coliforms, LAB, Clostridia*
Fish	Discoloration	*Pseudomonas*
	Putrefaction	*Chromobacterium, Halobacterium, Micrococcus*
Eggs	Green rot	*Pseudomonas*
	Colorless rot	*Pseudomonas, Alcaligenes, Chromobacterium*
	Black rot	*Coliforms*
	Fungal rot	*Proteus, Penicillium, Mucor*

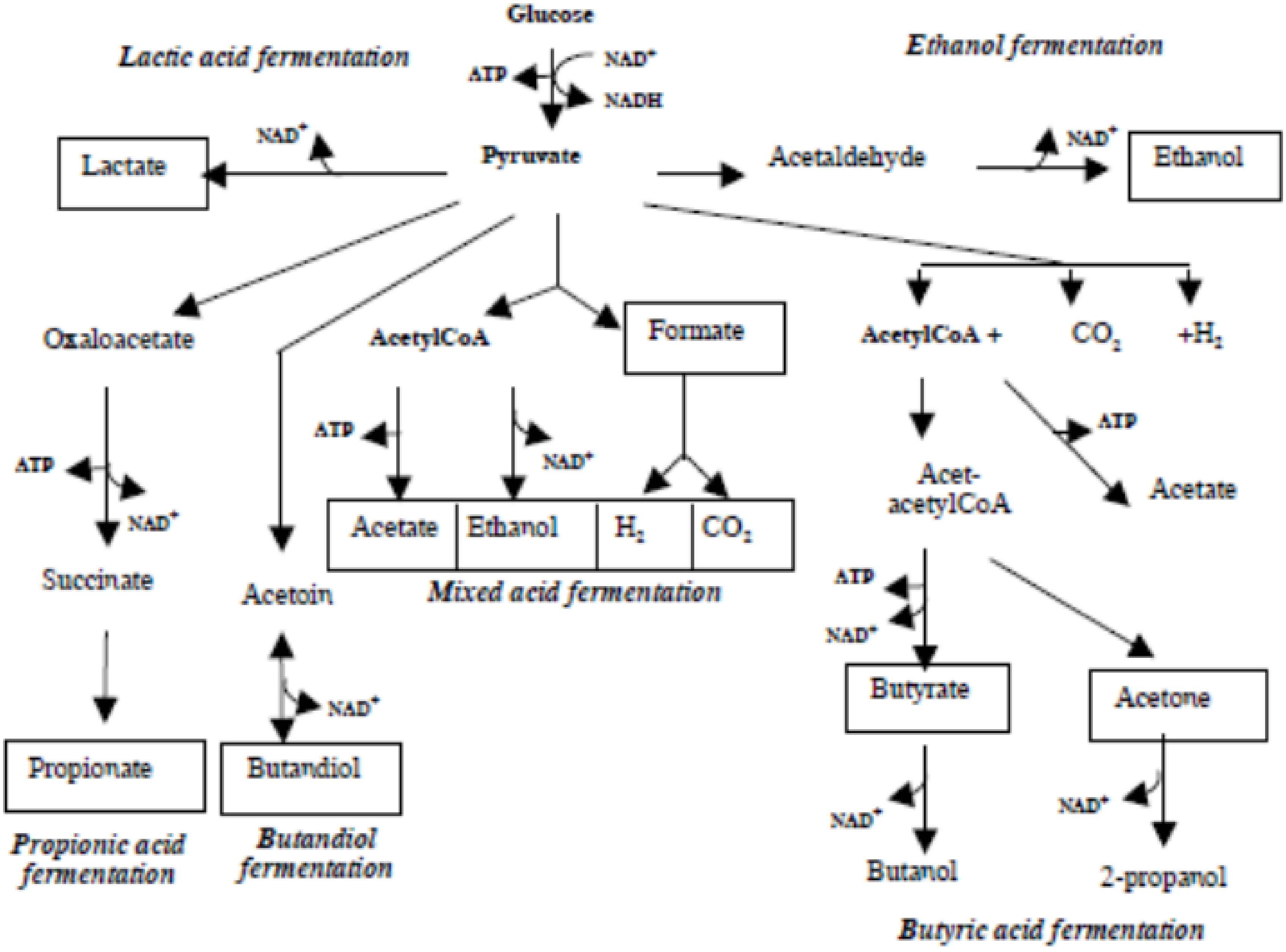
Food	Types of Spoilage	Spoilage Microorganisms
 **FRESH FRUITS AND VEGETABLES**	Bacterial soft rot	*Erwinia carotovera, Pseudomonas spp.*
	Gray mould rot	*Botryitis cinerea*
	Rhizopus soft rot	*Rhizopus nigrican*
	Blue mould rot	*Penicillium italicum*
	Black mould rot	*Aspergillus niger, Alternaria*
	Sliminess and Souring	*Saprophytic bacteria*
Canned food	Flat Sour	*Bacillus coagulans, B. sterothermophilus*
	Thermophillic acid	*Clostridium thermosacchrolyticum*
	Sulphide stinker	*Clostridium nigrificans*
	Butyric acid fermentation	*C. butyricum*
	Softening of fruits	*Byssochlamys fulva*
	Sliminess	Yeast and molds
Wine	Off Flavor, bitterness	Acetobactor, Oenococcus

Chemical changes caused by micro organisms

- **Degradation of carbohydrates**
- **Degradation of N- compounds**
- **Degradation of lipids**
- **Pectin hydrolysis**

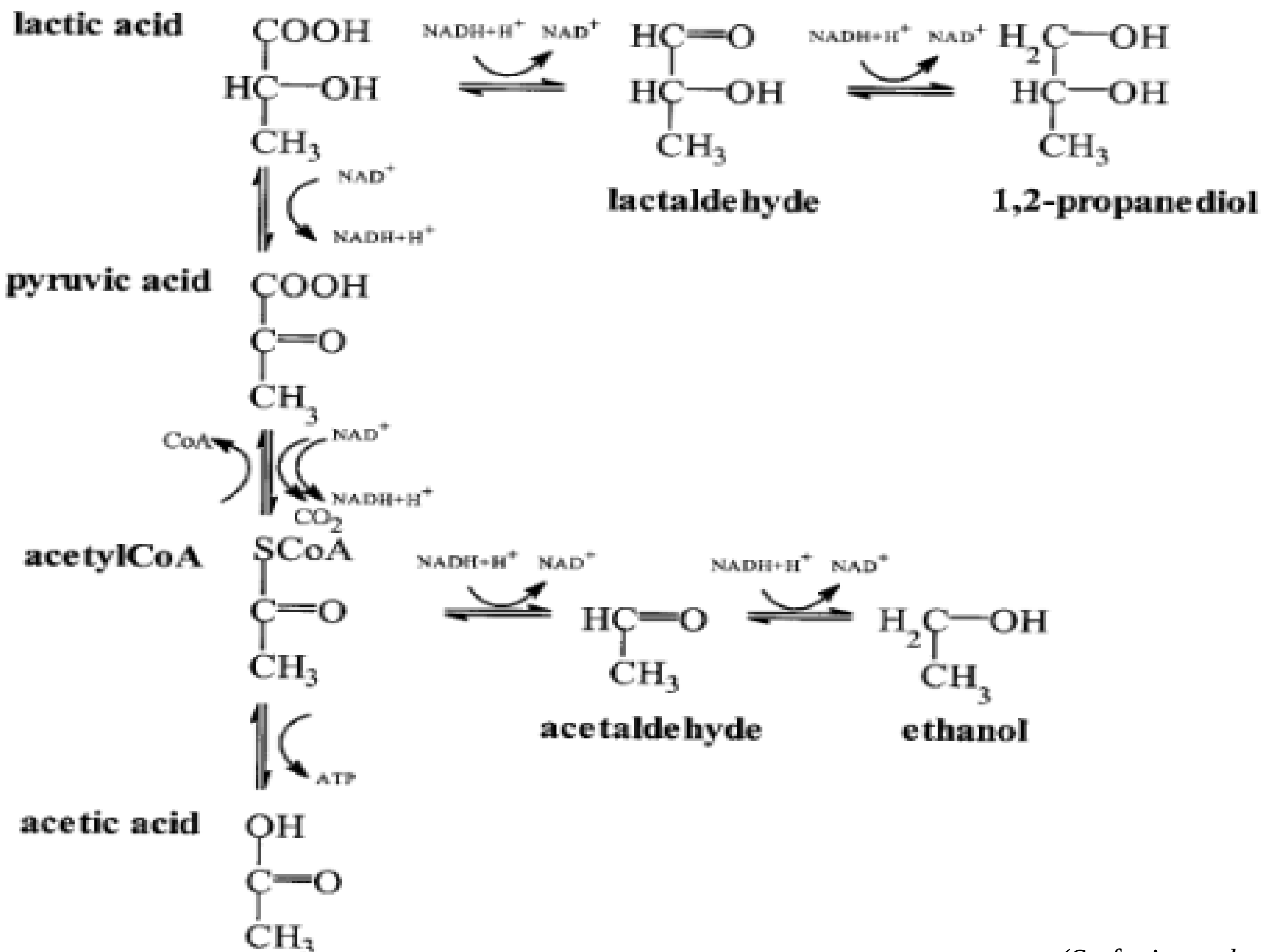
Degradation of carbohydrates

Fermentation type	Products
Alcoholic Fermentation	Ethanol, CO_2
Homofermentative lactic acid Fermentation	Lactic acid
Heterofermentative lactic acid Fermentation	Lactic acid, Acetic acid, Ethanol, CO_2
Propionic acid Fermentation	Propionic acid, Acetic acid, CO_2
Butyric acid Fermentation	Butyric acid, Acetic acid, $CO_{2,}$ H_2
Mixed acid Fermentation	Lactic acid, Acetic acid, $CO_{2,}$ $H_{2,}$ Ethanol
2,3-butanediol Fermentation	$CO_{2,}$ Ethanol, 2,3-butanediol , Formic acid



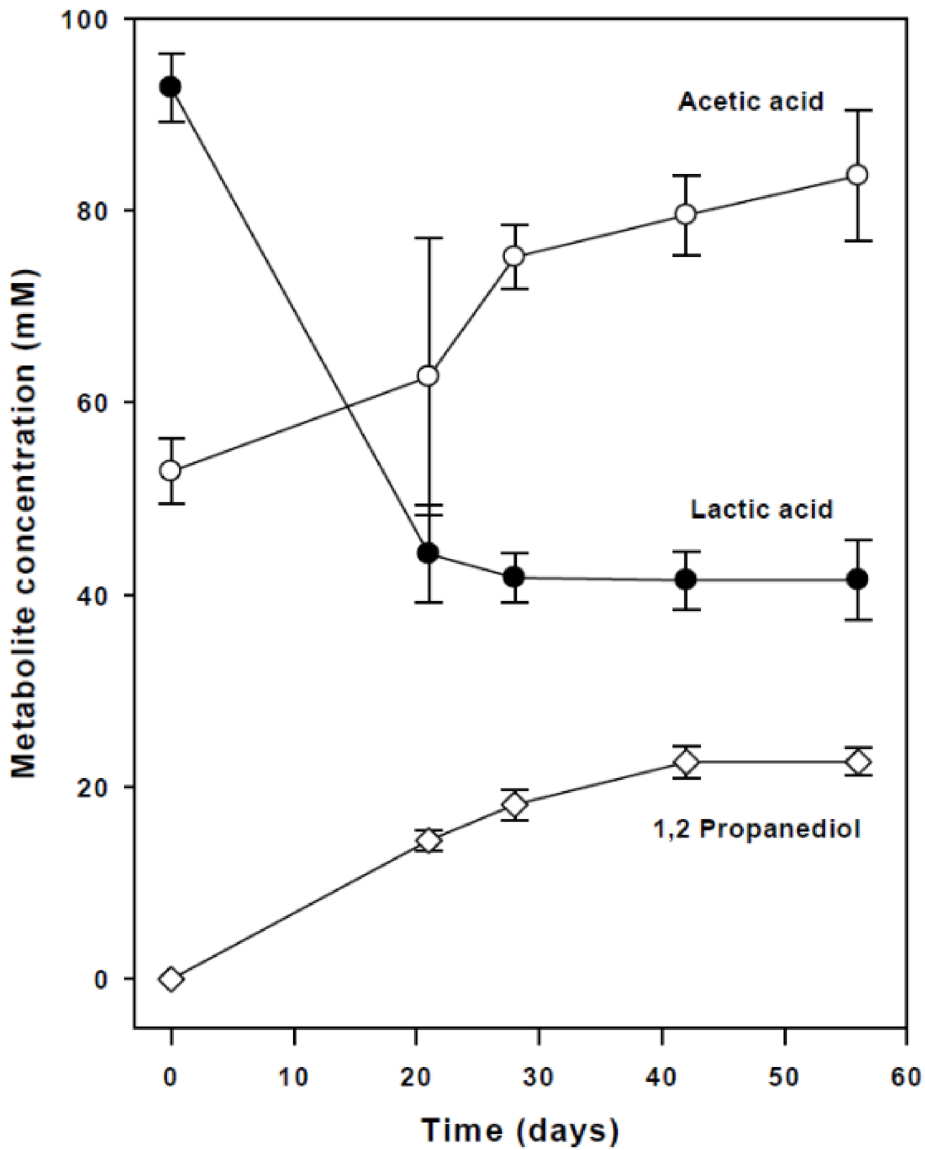
Glucose
Lactic acid fermentation
ATP
NAD+
NADH
Ethanol fermentation
NAD+
Lactate
Pyruvate
Acetaldehyde
NAD+
Ethanol
Oxaloacetate
AcetylCoA
Formate
AcetylCoA +
CO2
+H2
ATP
NAD+
ATP
ATP
NAD+
Acet-acetylCoA
Acetate
Acetate
Ethanol
H2
CO2
Succinate
Acetoin
Mixed acid fermentation
ATP
NAD+
NAD+
Butyrate
Acetone
Propionate
Butandiol
NAD+
NAD+
Propionic acid fermentation
Butandiol fermentation
Butanol
2-propanol
Butyric acid fermentation

Anaerobic Conversion of Lactic Acid to Acetic Acid and 1,2 Propanediol by *Lactobacillus buchneri*



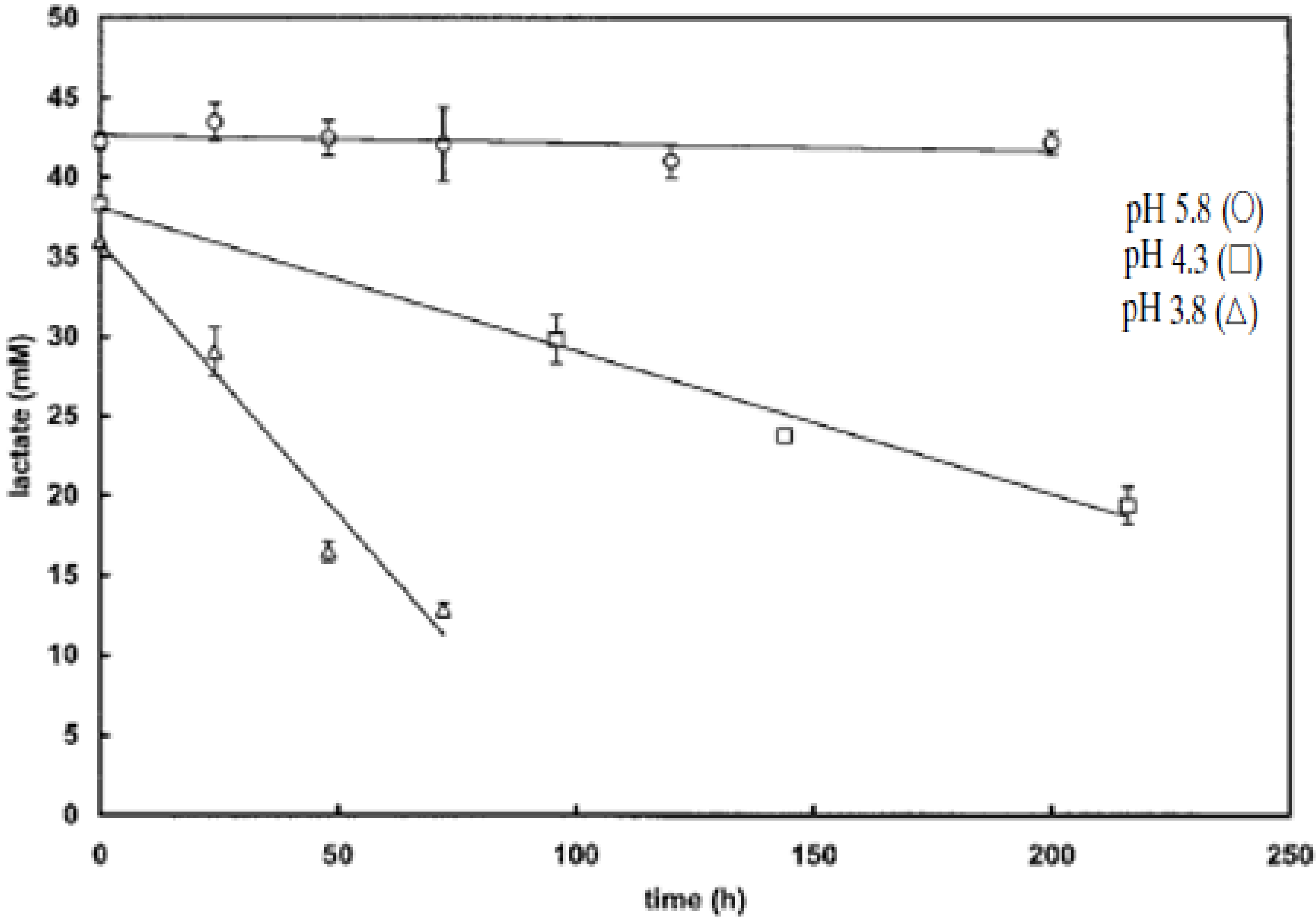
(Stefanie *et al.*, 2001)

Lactic acid utilization by *Lactobacillus buchneri*, a potential spoilage organism in fermented cucumbers



(Johanningsmeier , 2011)

Lactate utilization in time by *L. buchneri* at different pH



(Stefanie *et. al.*, 2001)

Degradation of N- compounds

Proteolysis

$$\text{Proteins} \xrightarrow{\text{Proteinases}} \text{Polypeptides} \xrightarrow{\text{Peptidases}} \text{Amino Acids}$$

Putrefaction

Amino Acids

Cysteine
Methionine
Tryptophan
Lysine
Arginine
Histidine

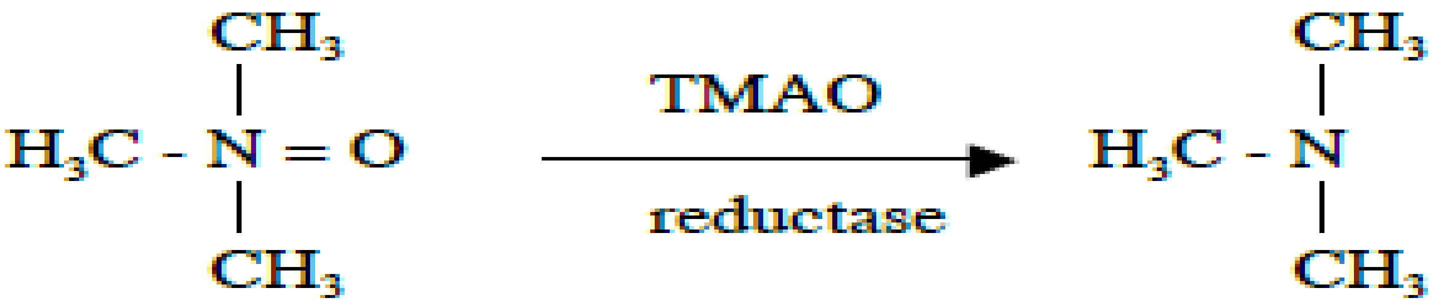
Bacterial Cell

Cysteine desulfhydrase
Methionine lyase
Tryptophanase
Decarboxylase

Volatile products

H_2S
Methyl mercaptans
Indole
Cadaverine
Putrescine
Histamine

Reduction of trimethylamine oxide



trimethylamine oxide

trimethylamine

Fishy odor

- **Pseudomonas**
- **Shewanella**
- **Bacillus**
- **Clostridium**

Degradation of lipids

$$\text{Lipids} \xrightarrow{\text{lipase}} \text{Glycerol + Fatty acid}$$

$$\downarrow \text{Lipid oxdase}$$

Aldehyde , ketones

- **Pseudomonas**
- **Micrococcus**
- **Staphylococcus**
- **Flavobacterium**

Pectin Degradation

Pectin → Polygalacturonic acid + Methanol

↓

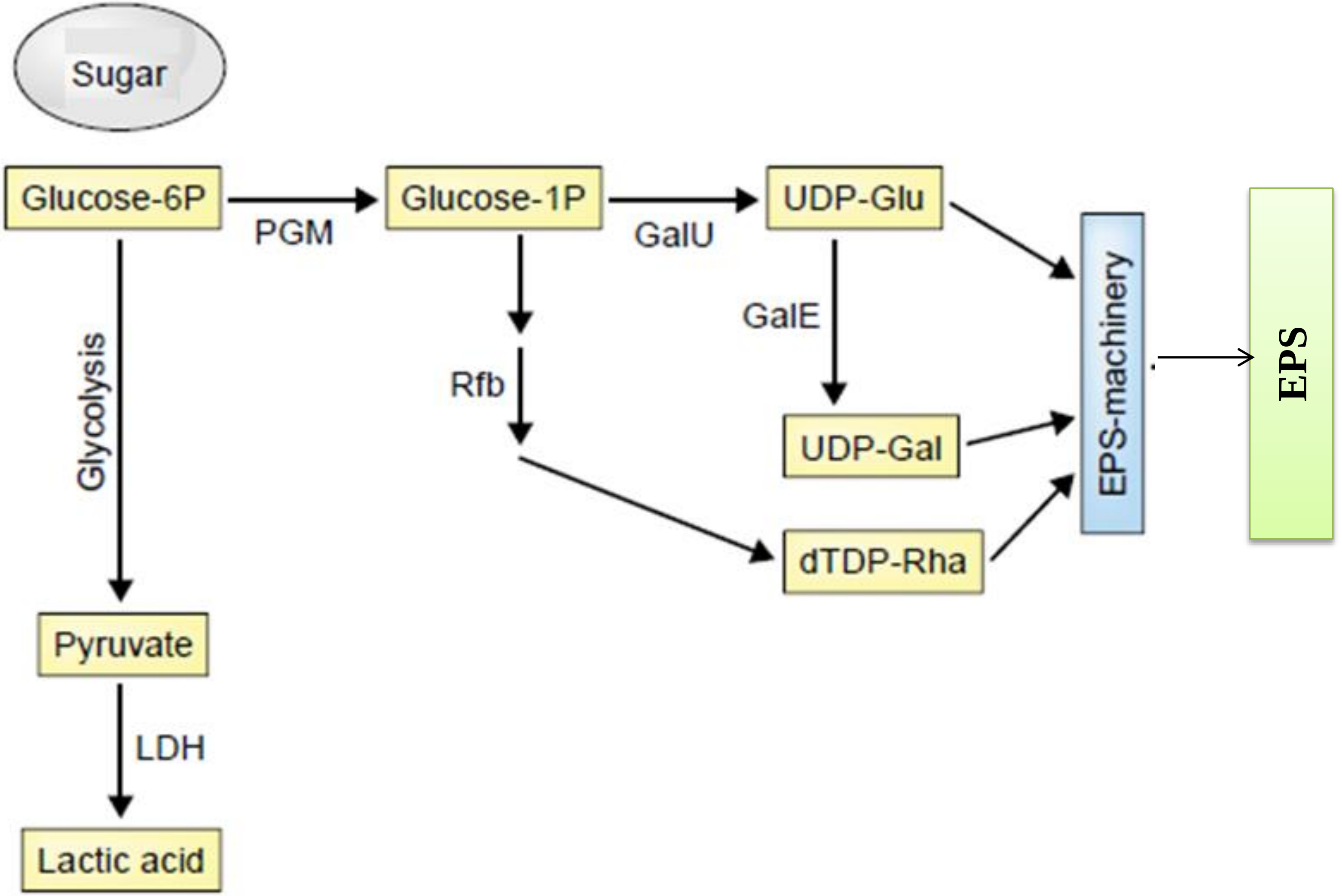
Galacturonic acid

Apple rot

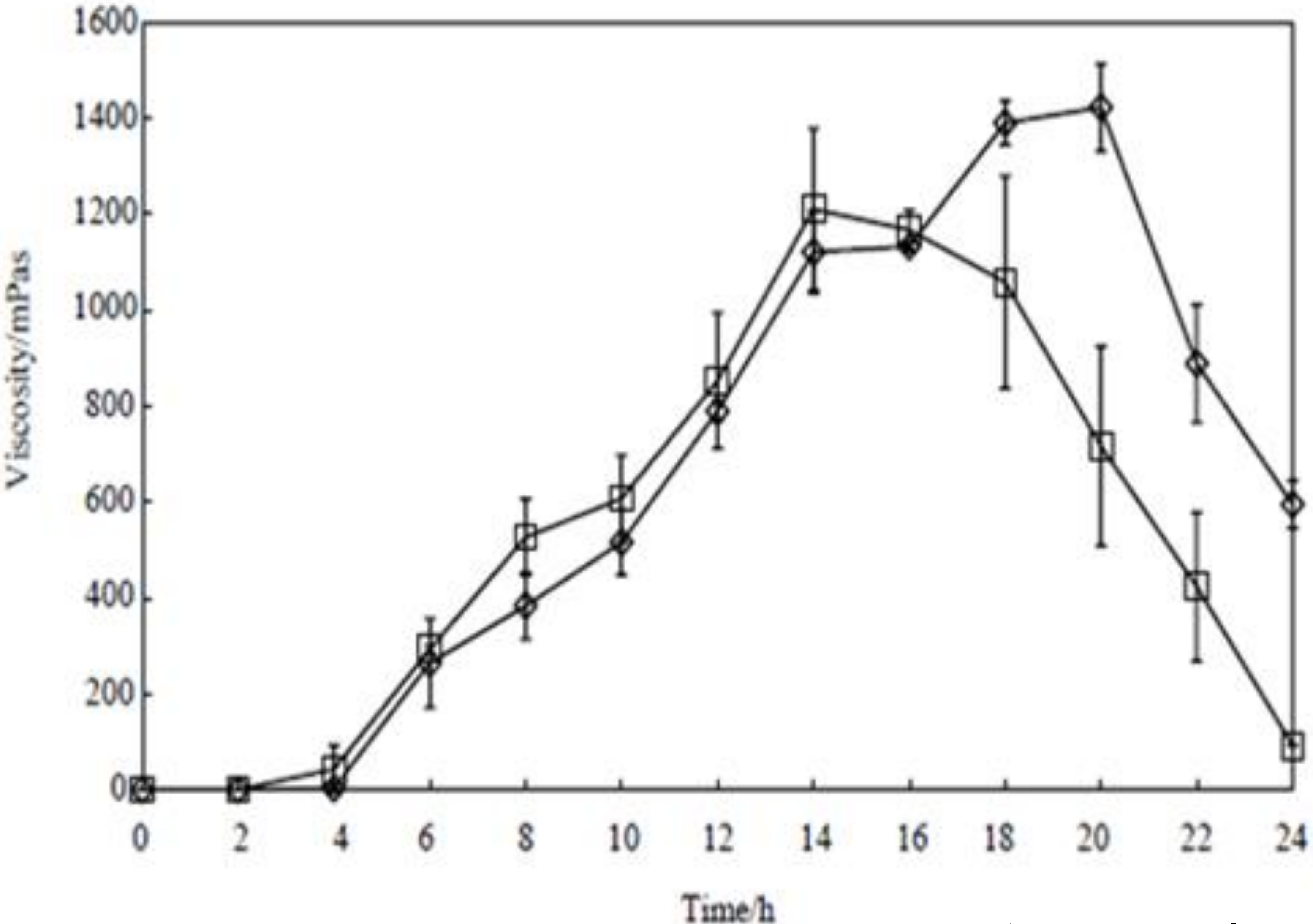
Penicillium expansum *Monilinia fructigena*

Soft and watery Dry and firm

Slime production

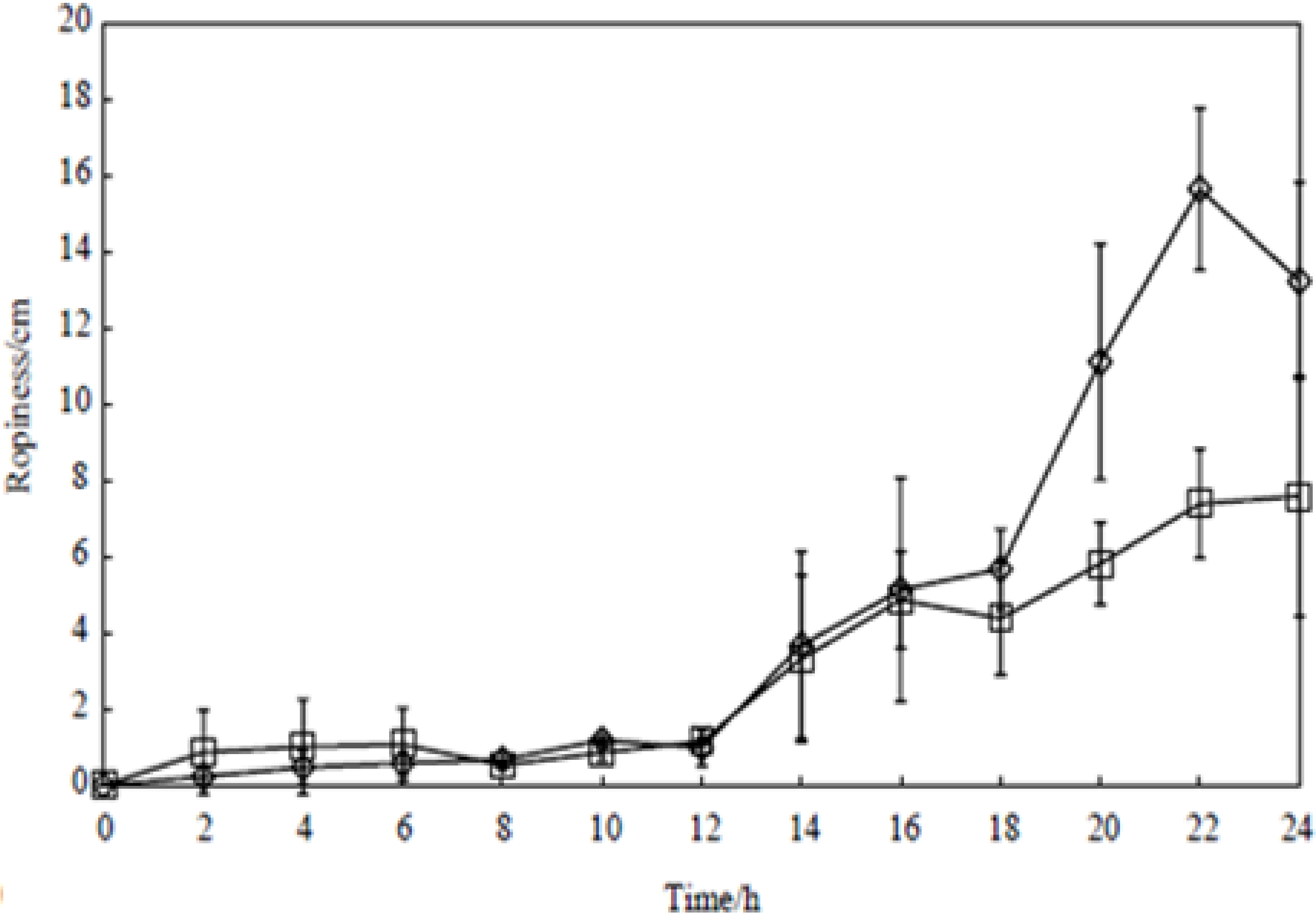


Viscosity of medium during growth of *Pediococcus damnosus* 2.6 (◊) and *Lactobacillus brevis G-77* (□) at 28°C for 24 h.



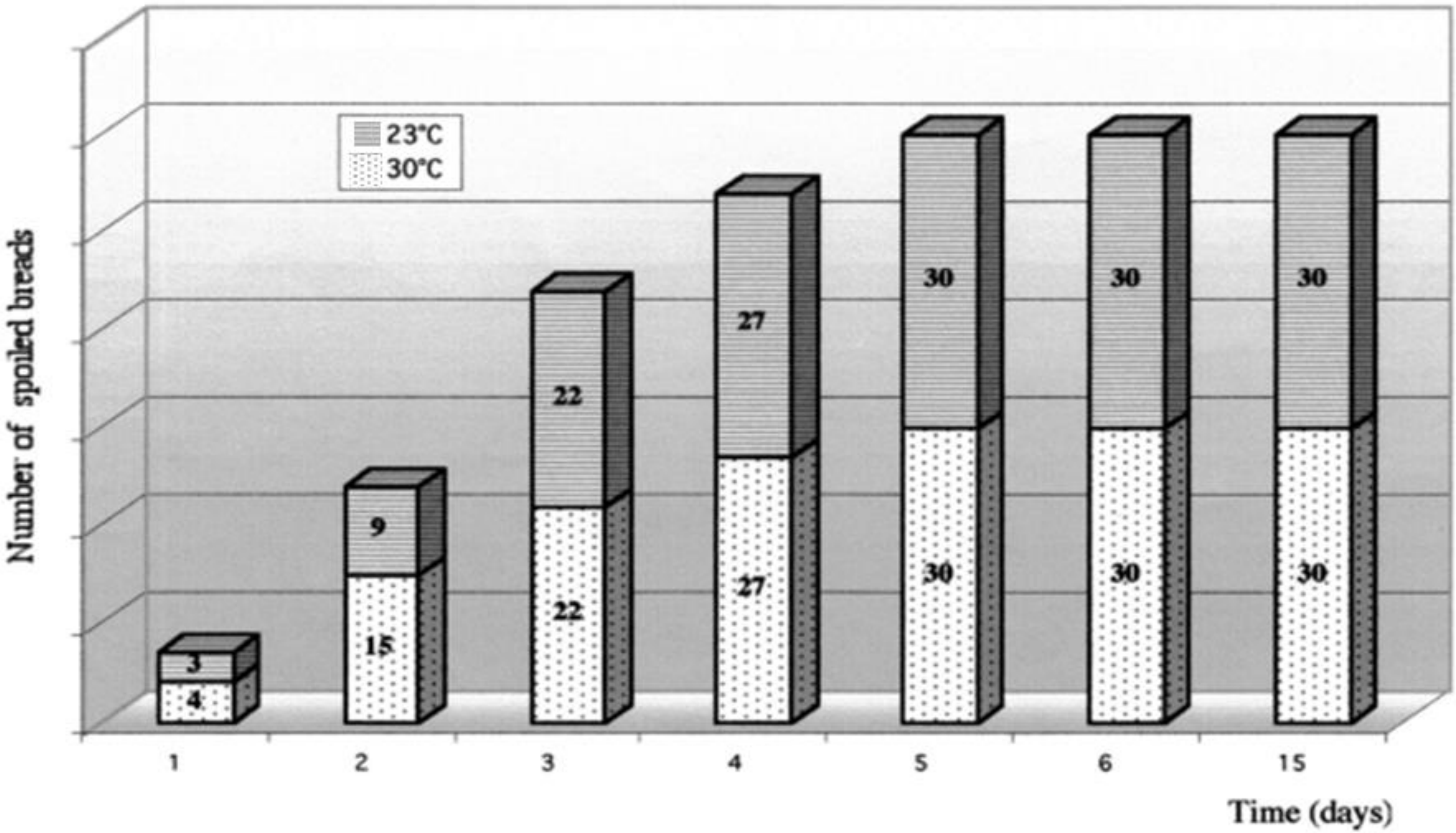
(Martenssona *et al.*, 2003)

Ropiness of medium during growth of *Pediococcus damnosus* 2.6 (◊) and *Lactobacillus brevis G-77* (□) at 28°C for 24 h



(Martenssona *et al*., 2003)

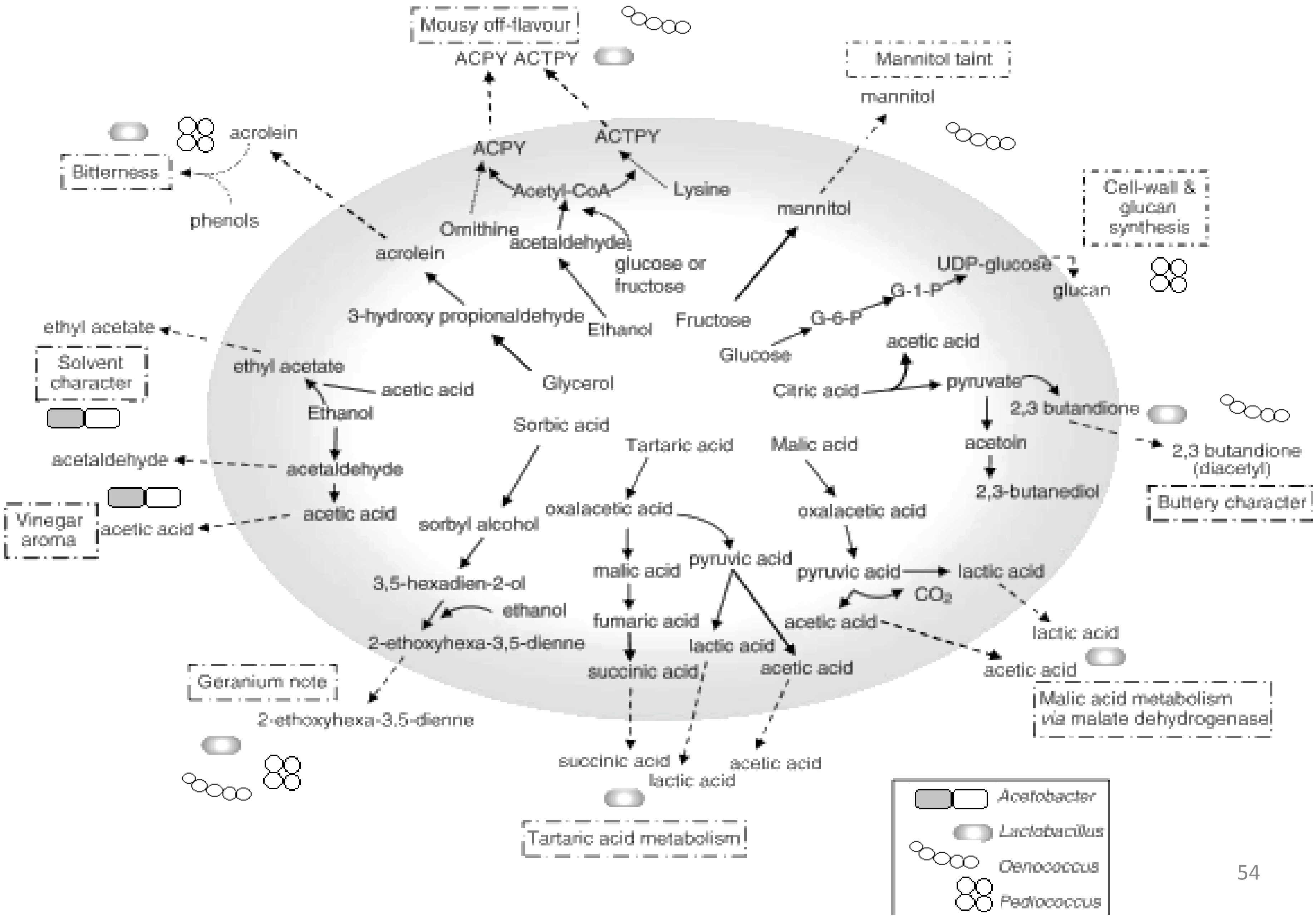
Rope-Producing Strains of *Bacillus* spp. from Wheat Bread



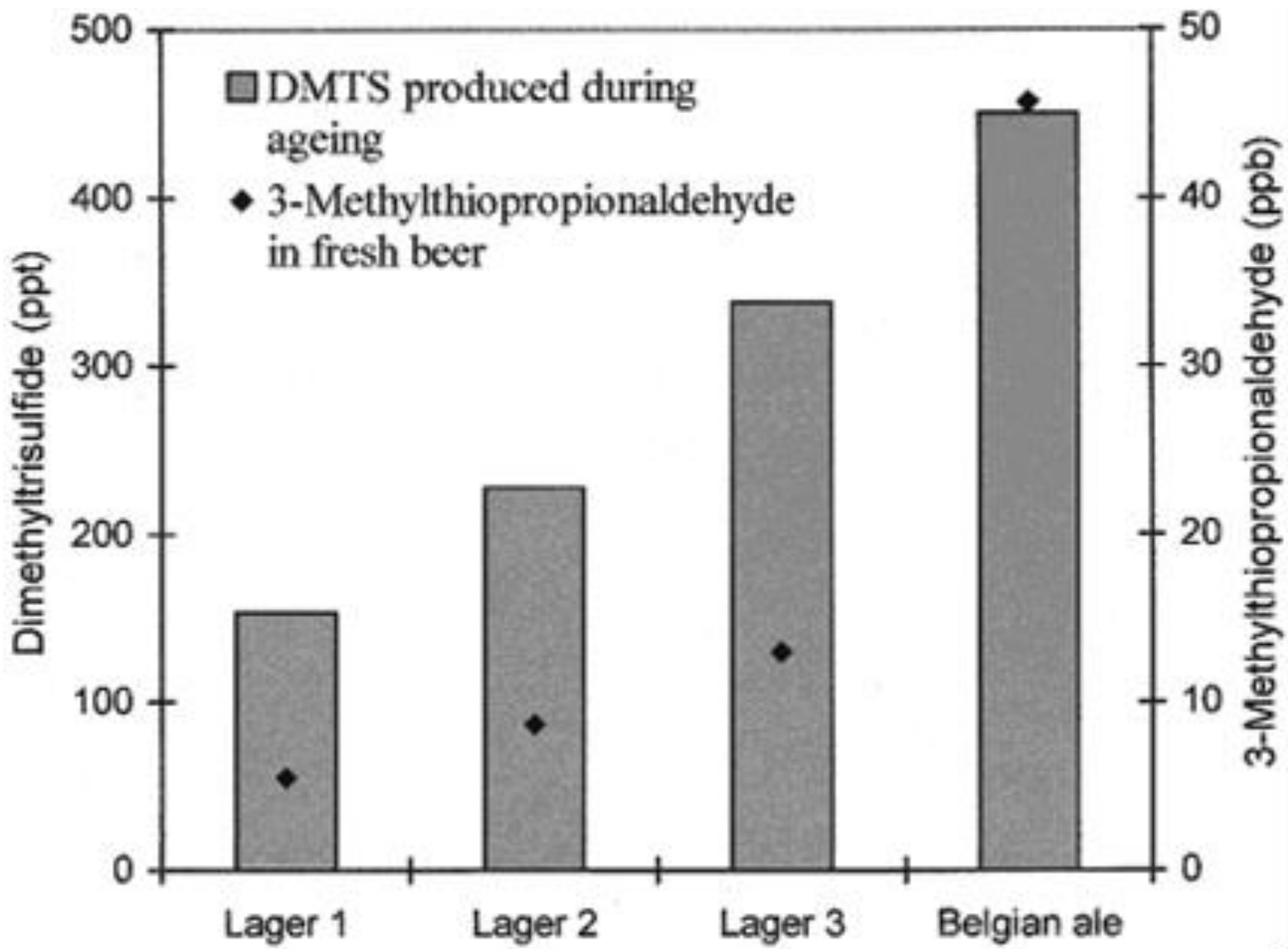
Total numbers of rope-spoiled breads during storage at 23 and 30°C

(Pepe, *et. al.,* 2003)

Summary of bacterial pathways leading to spoilage aroma and flavor compounds of wine



3-Methylthiopropionaldehyde as Precursor of Dimethyl Trisulfide in Aged Beers



(Gijs *et al.*, 2000)

Off flavor	Chemical compounds	Food
Fishy	Trimethylamine	Meat, egg, fish
Garlic Onion Cabbage	Dimethyl trisulphide Dimethyl disulphide Dimethyl sulphide	Wine, fish, meat, milk
Fruity	Esters	Milk, fish, wine
Potato	2-methoxy-3-isopropylpyrazine	Meat, egg, fish
Alcoholic	Ethanol	Fruit juices, mayonnaise
Musty odour	Trichloroanisole	Bread, wine
Cheesy odour	Diacetyl, acetoin	Meat
Medicinal odor	2-methoxy phenol	Juice, wine
Souring	Acetic acid, lactic acid, citric acid	Wine, bear, dairy

Texture problem	Chemical	Food
Slime	Polysaccharide	Meat, juices, wine, confectionery
Softening	Pectin degradation	Fruits and vegetable
Curdling	Lactic acid	Milk
Holes	Carbon dioxide	Hard cheese

Visual problems	Chemical	Food
Bloaters	Gas production	Fermented cucumber
Holes	Gas production	Hard cheese
Can swelling	Gas production	Canned foods

Prevention

- **By keeping out microorganisms**

- **By hindering the growth and activity of microorganisms**

    - Low temperature
    - Drying
    - Chemicals
    - Antibiotics

- **By killing the microorganisms**

Conclusion

Foods spoil due to physical, chemical and microbial degradation with their metabolites being the cause of the off-flavours or the textural changes resulting in sensory rejection.

These factors are interrelated, as certain temperatures and oxygen and moisture levels increase the activities of endogenous enzymes and of microbes.

Rodent and insect damage may provide an entry point for microbial growth.

Which microorganisms will develop or what (bio)chemical reactions occur is dependant upon food derived or environmental factors.

Conclusion

Although food spoilage is a major economical loss, the underlying integrated mechanisms are still poorly understood.

There is a need for the identification and control of growth of Specific spoilage organism (SSO) present on different food commodities. As yet not many SSO have been identified.

Therefore, the estimation of the quality of a food product still relies on the quantification of total numbers of microorganisms, which in some cases is a very poor reflection of the actual quality.

In addition to the identification of SSO, a better understanding of the complex interaction between SSO and other microorganisms or their metabolites is needed.

Finally the interaction between microbial spoilage and chemical spoilage has to be elucidated.

THANK YOU